APPENDIX A
SUPPORTING INFORMATION

A.1. DRINKING WATER SYSTEMS IN THE UNITED STATES

For the last 100 years, drinking water utilities in the United States have played a major role in protecting public health through the reduction of waterborne disease. The reductions in waterborne disease outbreaks were brought about through the use of sand filtration, disinfection, watershed management and the application of drinking water standards.

The United States has nearly 60,000 community water suppliers serving over 226 million people. Figure A.1-1 illustrates the distribution of water supply systems by type and source water. Nearly all of the utilities that use surface water practice some type of treatment and many practice what is called conventional treatment which is the primary focus here. Consequently, over 200 million people in the United States are routinely exposed to disinfected water and hence disinfection byproducts.

In 1974, chloroform was identified in disinfected drinking water. Additional halogenated compounds were found and classified as trihalomethanes, haloacedic acids, and haloacetonitriles. Each class of compounds can be represented by the examples in Figure A.1-2.

A.1.1. Objectives of Water Treatment. Drinking water treatment has three general objectives (Hudson, 1981):

- to remove any toxic or health-hazardous materials;
- to remove or inactivate any disease-producing organisms; and
- to improve the aesthetic acceptability of the water.

Good engineering practice requires that these objectives be achieved with a reasonable factor of safety and at reasonable cost.

Defining treatment objectives and selecting appropriate control technologies involves eight basic considerations:

- Effluent requirements
- Adequate quantity
- Influent characteristics
- Existing system configuration
- Cost
- Operating requirements
- Pre-treatment and post-treatment components
- Waste management

A.1.1.1. Effluent Requirements and Influent Characteristics — Effluent requirements are usually specified by drinking water standards and regulations as discussed earlier. Comparisons between influent characteristics and effluent requirements provide the basis for identifying treatment needs. The influent is properly characterized by a historical profile of the physical (i.e., time, turbidity) and biological constituents of the source water. Chemical parameters such as pH are also useful in characterizing the influent because they impact treatment process efficiency.

A.1.1.2. Costs — Treatment process costs are usually divided into capital, operating and maintenance costs. Frequently, unit processes with high capital costs are associated with low operation and maintenance costs. Smaller systems usually have higher unit costs because they do not benefit from economies of scale. Cost principles for drinking water treatment will be discussed in more detail later.

A.1.1.3. Operating Requirements — Consistency of the quality and volume of the influent is a critical operating factor. If the influent is highly variable, increased monitoring and operator skills are required to maintain acceptable quality.

A.1.1.4. Pre-Treatment and Post-Treatment Processes — Water treatment processes perform differently with different pre- and post-treatment processes. The compatibility of all unit processes in the treatment train is important in achieving individual treatment goals. For successful implementation of a treatment technology, all elements of the treatment train must interact efficiently and effectively.

A.1.1.5. Waste Management — Waste management is a major concern associated with the removal of contaminants in drinking water. Most conventional treatment processes concentrate contaminants into sludge that frequently requires special handling. Extensive regulations cover the residuals that result from water treatment operation.

A.1.2. Water Treatment Unit Processes. Figure A.1-3 shows source water contaminants, chemicals added during treatment, some of the treatment byproducts, and some of the associated waste streams. Figure A.1-4 depicts a conventional treatment train. The standard unit processes that make up conventional treatment are chemical feed, rapid mix, flocculation, sedimentation, filtration and disinfection. Each of the unit processes shown in Figure A.1-4 is discussed in the following sections.

A.1.2.1. Chemical Addition — The three types of chemicals usually applied to the raw water at the beginning of the conventional treatment train are coagulants, coagulant aids, and pH control substances. Coagulants are chemicals used to remove turbidity and organic substances from raw water by precipitation. Coagulant aids are added to the influent after or simultaneously

with the primary coagulant to improve flocculation performance. The optimum pH range favors insolubility of metallic coagulants, improves the strength of the floc and enhances turbidity removal through sedimentation and filtration. Disinfectants such as chlorine are sometimes used at the front end of the treatment train, although in order to minimize the formation of disinfection byproducts, it is frequently added at the end of treatment. More recently, ozone application as a pre-treatment has been used to oxidize some organics to lower molecular weight compounds, thus reducing byproduct formation.

A.1.2.2. Coagulation/Rapid Mix — Rapid mixing is generally the first stage of the treatment process. It is essential that complete dispersion and rapid mixing of the coagulant and raw water be accomplished in order to effect maximum turbidity removal. While some plants only have one rapid mixer to add coagulants, other plants have multiple rapid mixers to achieve optimum performance.

A.1.2.3. Flocculation — Flocculation usually follows the rapid mixing step in conventional treatment plants. It provides multiple opportunities for particles suspended in water to collide and aggregate through gentle and prolonged agitation. This process takes place in a basin equipped with a low-speed mixer.

A.1.2.4. Sedimentation/Clarification — Sedimentation and clarification is the step that follows flocculation and precedes filtration. Its purpose is to enhance the filtration process by removing particulates that were carried over from the flocculation process. Sedimentation requires that water flow through the basin at a velocity slow enough to permit the particles to settle to the bottom before the water exits the basin.

A.1.2.5. Filtration — Filtration is the process of passing the settled water through a porous medium to remove fine particulate matter remaining in suspension. In water purification, the matter to be removed is usually colloidal in size and includes suspended silt, clay, organic colloids, and microorganisms, including algae, bacteria, viruses and protozoa.

A.1.2.6. Disinfection — Disinfection is a process that kills or inactivates microorganisms, including most waterborne pathogens that occur in drinking water. The most common disinfectant used in the United States is chlorine. Other types of disinfectants are chloramines, chlorine dioxide and ozone.

A.1.2.7. Overall Treatment Train — Figure A.1-5 shows a cross-section of a complete treatment train. This arrangement of unit processes is considered good engineering practice and used extensively throughout the United States. There are several possible modifications to traditional filtration systems including direct filtration, slow sand filtration, package plants, diatomaceous earth filtration, membrane filters and cartridge filters. These modifications are not discussed in this document.

A.1.2.8. Efficiency in Microbial Treatment — Conventional water treatment, including disinfection, is generally efficient in controlling the penetration of microorganisms into the distribution system. For example, Hurst (1991) has shown that conventional treatment with post-disinfection is quite effective in controlling viruses. According to Hurst, the overall efficiency of coagulation and sedimentation at removing viruses was 50.4%. The median values for virus removal efficiency during subsequent stages in the treatment sequence were: filtration, 72.1%; post-filtration disinfection, >45.0%. The median efficiency for the combination of coagulation and sedimentation followed by filtration was 86.2%. When post-filtration

disinfection is added, the efficiency increases to >95.1%. Addition of pre-disinfection can increase the efficiency to 99.98% for enterovirus removal.

Even greater removal efficiencies may be obtained for bacteria, but if treatment modifications are made, these efficiencies may change. For example, changing the site of chlorine application may allow for migration of organisms deeper into the treatment train. Geldreich (1993) examined the results from moving the point-of-chlorination from pre-chlorination to post-chlorination at the Cincinnati Water Works. It was found that the concentration of bacteria changed with the location of the disinfectant.

A.1.3. Distribution System Issues. Although it is most common to think in terms of risks associated with treatment plant effluents, water quality can deteriorate as it moves through the distribution system. For example, disinfection byproducts generally increase with time in the distribution system. Therefore, data from simulated distribution studies have been analyzed in conjunction with the pilot plant data mentioned previously to simulate this effect.

A.1.4. Water Treatment Performance Data. The promulgation of the Information Collection Rule (ICR) and proposals for a Disinfectant and Disinfection Byproducts Rule (D-DBP) and Enhanced Surface Water Treatment Rule (ESWTR) have increased interest in treatment techniques that can minimize DBPs and remove DBP precursors while at the same time enhancing disinfection. Ozonation, although not a new technology, may assist drinking water utilities in meeting these goals. Ozone is a strong disinfectant and has the capability of oxidizing DBP precursors. Limited experimental evidence indicates that ozone may be effective in inactivating *Cryptosporidium* oocysts (Miltner et al., 1997; Finch et al., 1993). It also produces some DBPs such as aldehydes and ketoacids and may increase bacterial nutrients by converting

nonbiodegradable organic matter into biodegradable compounds. This conversion may, in turn, lead to microbial regrowth in the distribution system, which is a major concern to all drinking water utilities. There is also evidence that ozone applied in concentrations that will be effective against *Cryptosporidium* oocysts will also enhance the formation of bromate, which may be regulated under the D-DBP Rule (Miltner et al., 1992, 1997; Krasner et al.,1993; Shukairy et al., 1994).

Under the proposed D-DBP Rules, the maximum contaminant level (MCL) for total trihalomethanes (TTHMs) would be lowered and brominated and haloacetic acids (HAAs) would be regulated. Aldehydes and other ozone-related DBPs may be regulated in the future. One strategy that has been suggested to minimize the negative aspects of ozone is to allow some of the filtration processes in the treatment plant to become biologically active, and use that microbiological population on the filters to incorporate the organic fragments and byproducts as nutrients.

In order to examine some of these options, studies were performed in the U.S. EPA's water treatment pilot plant in Cincinnati, Ohio. During these studies, ozone was used as a pre-disinfectant and a comparison of the effect of using ozone versus conventional treatment (no pre-disinfection) on DBP formation and control was evaluated. Both treatment configurations used post-chlorination application in the clearwell of the pilot plant.

A.1.4.1. Pilot-Scale Treatment Plant — Figure A.1-6 shows the unit process arrangement of EPA's pilot plant as it was configured for this study. This study and its operational conditions are described in detail elsewhere (Miltner et al., 1990). For the study, raw

Ohio River water was trucked to the U.S. EPA and treated at 1.7 gpm. Ozone was applied so that the transferred ozone/TOC ratio was near 0.8.

Previous studies have shown that at this ratio, optimal oxidation of trihalomethane formation potential (THMFP), haloacetic acid formation potential (HAAFP) and total organic halide formation potential (TOXFP) would occur, conversion of bromide to bromate would be minimal, and the production of aldehydes, AOC and BDOC would not have reached their maximum values (Miltner et al., 1992). Design loading rates and EBCTs for the filters for both treatment trains were 2 gpm/ft^2 (81.5 L/min/m^2) and 9.2 min (30-inch media depth), respectively.

Chlorine was applied in the clearwell after filtration to yield a free residual near 0.2 mg/L in samples taken from the clear wells and stored 3 days to simulate distribution. Chlorine doses were in the range of 2.8-3.0 mg/L resulting in free chlorine residuals in clear well effluents near 1.2 mg/L. Detention time in the clear wells was approximately 9.5 hours.

On the first day of the study, new media was placed in each filter at its design depth, backwashed with Cincinnati tap water to remove fines, and placed in service. Figure A.1-5 shows sampling locations utilized during the study (solid dots). Both instantaneous and terminal DBPs were determined. The methods employed for data presented in this paper were taken from Standard Methods or EPA methods. Table A.1-1 describes the pilot-scale operation of the two treatment trains.

A.1.4.2. Microbial Performance Data — Data on the removal of the target organism *Cryptosporidium* was obtained from the literature and is reported in Table A.1-2. Table A.1-2 summarizes *Cryptosporidium* oocyst removals for conventional treatment with post-chlorination.

TABLE A.1-1

Mean Pilot Plant Operation by Unit Operation

	Raw	Ozone	Alum		Chlorine		Finished	
			O_3-Cl_2	Cl_2	O_3-Cl_2	Cl_2	O_3-Cl_2	Cl_2
O_3 dose, mg/L		1.93						
Alum dose, mg/L			15.4	15				
Cl_2 dose, mg/L					2.82	3.04		
pH	7.92						7.69	7.72
Turbidity, ntu	14.8						0.15	0.18
Temperature, ^oC	27.8						26.7	26.7
Free Cl_2, mg/L							1.20	1.14
Total coliform, CFU/100 mL	54,600						< 1	< 1
TOC, mg/L	2.41						1.76	1.98

TABLE A.1-2

Cryptosporidium and *Giardia* Removal by Conventional Treatment with Post Chlorination[a]

Microbe Density/L- GM		Log Removal
Raw Water	Finished Water	
0.14 oocysts (<1 - 100)[b]	<0.1 oocysts	(3.2)
2.00 cysts (20 - 1000)[b]	<0.1 cysts	(4.3)

[a] Source: Payment et al., 1997
[b] interpreted from graph

A.1.5. Cost Analysis for Base Scenarios. The flow diagrams for the two full-scale treatment trains considered in this analysis are shown are shown in Figures A.1-7 and A.1-8 and the unit process cost curves are contained in Appendix A. Table A.1-3 contains the assumptions underlying the analysis and Table A.1-4 summarizes the costs associated with conventional treatment with and without pre-ozonation.

A.1.5.1. Discussion — A major issue raised when considering the use of chlorine for treating drinking water is the exposure from potentially carcinogenic products of disinfection versus the effectiveness of chlorine for protection against microbial infection. Therefore, alternative treatment techniques such as ozone are being considered. It is well established that the application of ozone increases the concentration of compounds such as methyl glyoxal and AOC-NOX, but based on the results of the pilot plant studies, it can be seen that these increases can be controlled by allowing the filters in the treatment train to become biologically active. THMFP is removed through the various treatment steps, with the biologically active filter removing slightly more THMFP than the conventional filter. HPCs increase through both trains but are easily controlled by chlorination. In both treatment trains, THMs increase at the chlorination step only.

Ozone is a powerful oxidant and when used in combination with a disinfectant such as chlorine can be very effective for water treatment. Based on the results of the analysis shown in this paper, when the filters are allowed to become biologically active, the negative aspects of using ozone seem to be mitigated. Byproducts are minimized and microorganisms are controlled.

TABLE A.1-3

Assumptions for Cost Analysis

Construction Cost and Producer Price Indices used to Update Costs to 1997		
Base Year	CCI	PPI
1979	265.4	199.7
1990	445	345
1997	549	361

Assumptions for Cost Analysis	
Item	Value
Capital cost amortization	10% interest for 20 years
Labor cost	$15/hr
Electric power cost	$0.08/kWH
Diesel	$1.25/gallon
Operating capacity	70% of design capacity

TABLE A.1-4

Cost of Conventional and Pre-Ozone/Conventional Water Treatment

Annual Costs for 130 mgd Conventional Water Treatment Plant		Incremental Cost of Ozone	
Item	Value	Item	Value
Capital amortized	$9,185,832	Capital amortized	$566,135
Energy	$5,189,203	Energy	$411,470
Materials	$209,600		
Labor	$1,138,886		
Chemicals	$833,488		
Total annual	$16,557,010	Total annual	$977,835
Cost ¢ / 1000gal	49.85	Cost ¢ / 1000gal	2.94

Using data from two pilot plant configurations and based on studies conducted in the pilot facilities at the U.S. EPA, a matrix of disinfection byproducts for these configurations has been generated. Full-scale equivalents of these pilot plants were hypothesized as shown in Figures A.1-6 and A.1-7. Unit costs were estimated for the two base case scenarios and these data were utilized to calculate cost and benefits associated with exposures to disinfection byproducts from alternative treatment processes.

A.2 WATERBORNE PATHOGENS

A.2.1. Introduction. A total of 672 reported waterborne disease outbreaks occurred during the time period 1946-1980 and affected over 150,000 people (Lippy and Waltrip, 1984). Outbreaks due to *Salmonella, Shigella, Campylobacter, Hepatitis A, Giardia,* Parvovirus-like agents, *Poliovirus, Pasteurella, Leptospira, Escherichia coli* and *Entamoeba* and acute gastroenteritis of unknown etiology (AGI) were reported during this time period. Descriptive information related to waterborne disease outbreaks reported in the U.S. during 1985-1994 are listed in Appendix A (Section A-2, Table A-2-1).

It is important to note here that surveillance data should be interpreted carefully, because most outbreak reporting is voluntary and depends upon public awareness, physician interest, laboratory testing facilities and expertise, and the level of effort of state and local surveillance activities. Data that identify water sources, water treatment and distribution systems and their deficiencies, and etiologic agents associated with disease outbreaks are needed in order to better evaluate the efficacy of water treatment technologies and to establish research priorities in support of water-quality regulations (CDC, 1996a).

A.2.2. Microbial Pathogens. Waterborne infections result from direct or indirect consumption of microbially contaminated water. Control of human disease depends on wastewater treatment or remediation to reduce the amount of pathogens present in or from contaminating drinking water supplies. Human and animal reservoirs exist for many pathogens found in water, and large quantities of enteric organisms from human and animal waste can be released into unprotected surface or ground water sources. Microbial communities can survive and even flourish in the relatively harsh environmental conditions of contaminated or polluted source waters.

TABLE A-2-1

Waterborne Disease Outbreaks Associated with Drinking Water and Etiologic Agent for the United States, 1985-1994.

YEARS	INFECTIOUS AGENTS	TOTAL # OF OUTBREAKS	# OF CASES/DEATHS	DRINKING WATER	REFERENCE
1985[a]	*Giardia lamblia*	3	741	chlorinated but unfiltered water	CDC 1988
	Campylobacter jejuni	2	169	repair of water main and untreated spring water	
	Shigella sonnei	1	27	untreated well water	
	Salmonella typhi	1	60	cross-contamination	
	AGI[b]	8	533	wells and springs	
1986-1988	*Cryptosporidium*	1	13,000	chlorinated and filtered water	CDC 1990
	Giardia	9	1,169	chlorinated but unfiltered surface water or other deficiency	
	Shigella sonnei	4	2,733	contamination of reservoir after heavy rains, deficiency of water supplies leading to contamination with sewage	
	Salmonella newport and other species	2	70	possible contamination of well water by sewer line and untreated well water	
	Campylobacter	1	250	water mains cross-connection contamination	
	Norwalk-like viral agent	3	5,474	multistate outbreak due to contaminated ice made from wells flooded by creek during heavy rains	
	AGI	24	2,982	surface and ground water	
	CGI[C]	1	72	untreated ground water	
1989-1990	*Giardia lamblia*	7	697	chlorinated but unfiltered water and untreated river water	CDC, 1991

YEARS	INFECTIOUS AGENTS	TOTAL # OF OUTBREAKS	# OF CASES/DEATHS	DRINKING WATER	REFERENCE
	Escherichia coli 0157:H7	1	243/4	distribution network and lack of disinfection at points of repair or replacement	
	Cyanobacteria (possible)	1	21	open air rooftop storage tanks	
	Hepatitis A	2	25	chlorinator malfunction and untreated well water	
	Norwalk-like agent	1	900	sewage effluent seeping into well	
	AGI	14	2,402	largest outbreak of 1000 cases due to well water within 20 ft of a septic tank	
1991-1992	*Giardia lambia*	4	123	unfiltered surface water, chlorinator not consistently maintained, and contaminated ground water	CDC 1993e
	Cryptosporidium	3	3,551	disinfected spring water , inadequate filtration of river water, and well water (finished water in all 3 outbreaks was free of coliforms and chlorine levels were probably sufficient to inactivate bacteria but not oocysts)	
	Shigella sonnei	1	150	untreated spring water contaminated with surface water	
	Hepatitis A	1	10	untreated well water	
	AGI	23	13,367	Largest outbreak of 9,847 cases due to water treatment deficiencies following a drought	
1993-1994	*Cryptosporidium parvum*	5	>403,271/58[d]	Largest outbreak in Milwaukee associated with water from state-of - the art treatment facility (cryptosporidiosis-associated deaths occurred from April 1, 1991-March 31, 1995)	CDC 1996a

YEARS	INFECTIOUS AGENTS	TOTAL # OF OUTBREAKS	# OF CASES/DEATHS	DRINKING WATER	REFERENCE
	Giardia lamblia	5	385	chlorinated but unfiltered surface water, cross connection between potable and waste water lines, filtered and chlorinated well water contaminated with sewage	
	Salmonella typhimurium	1	625	bird droppings in inadequately protected storage tower	
	Campylobacter jejuni	3	223	untreated well water contaminated in storage tower	
	Shigella sonnei	1	230	well water	
	Shigella flexneri	1	33	private home well water	
	Non-01 *Vibrio chloerae*	1	11	bottled water, contamination source unknown	
	AGI	5	495	wells	

[a] No waterborne outbreaks of documented viral disease reported in 1985
[b] AGI Acute gastrointestinal illness of unknown etiology
[c] CGI Chronic gastrointestinal illness of unknown etiology
[d] Hoxie et al 1997 follow-up. There were 58 *Cryptosporidium*-associated deaths From April 1, 1991 through March 31, 1995 among residents of the Milwaukee vicinity. Four deaths occurred before the outbreak period March 15, 1993-March 31, 1995. AIDs was the underlying cause of death for 85% (46/54) of the postoutbreak *Cryptosporidium*-associated deaths. Some caution should be applied to these estimates (e.g., 4 AIDs cryptosporidiosis-associated deaths in the 2 year preexposure period and 54 AIDs *Cryptosporidium*-associated deaths in the 2-year postexposure period. Four deaths would be expected and 50 additional deaths occurred. Some of the decedents could have been infected with *Cryptosporidium* elsewhere at a different time or from a different source. Also because of the increased awareness of cryptosporidiosis during the outbreak period, there was increased likelihood that cryptosporidiosis would be listed as cause of death. (An increased awareness would not represent a true increase in occurrence.) Other observations suggest that there was premature AIDs mortality during the first 6 months after the outbreak, followed by 2, 6 month periods with lower-than-expected AIDs mortality, suggesting that there may have been some underreporting of cryptosporidiosis as cause of death in persons with AIDs. The authors conclude that a more precise estimate of *Cryptosporidium*-associated deaths would require more studies.

Microbial survival and propagation is dependent on the availability of nutrients, water temperature, pH, chemical pollutant levels, runoff, sediment, and other environmental characteristics.

Bacterial pathogens (e.g., *Salmonella, Shigella, Escherichia coli,* and *Vibrio cholerae*) associated with contaminated source waters are readily inactivated or removed by routine water treatment that includes sedimentation, flocculation, filtration, and disinfection (e.g., chlorination). Although virus tend to be more persistent than bacteria in aquatic environments, and are more resistant to chlorination, they are still generally eliminated or inactivated by routine water treatment (Clark, 1998 personal communication).

Intestinal cyst-forming protozoan parasites, such as *Cryptospoidium parvum, Giardia lamblia, Isospora belli, Cyclospora cayetanensis, Enterocytozoan bieneusi* and *Septata intestinalis* are transmitted by the fecal-oral route through contaminated water or food, and by secondary spread (Rose, 1993). The epidemiology of these agents is not clearly understood, but they are frequent AIDS related pathogens and have been implicated in traveler's diarrhea (Jokipii and Jokipii 1986; Sterling et al., 1986; Ma et al., 1985), institutional and community outbreaks of diarrhea, and acute sporadic childhood gastroenteritis (Goodgame, 1996).

Giardia, *Entamoeba* and *Cryptosporidium* are currently the primary protozoa of concern in U.S. drinking water (Rose, 1993). However, waterborne *Entamoeba* outbreaks are rare in the United States and none have been known to occur since 1971 (Rose, 1993). *Giardia* has frequently been implicated in waterborne disease outbreaks, (See Table A-2-1) but most cases are asymptomatic and are rarely fatal (Rose, 1993), and several effective therapeutic agents are available for treatment. *Cryptosporidium* can cause severe protracted illness and death in those

with compromised immune systems (Flanigan et al. 1992; McGowan et al. 1993). There are currently no effective therapeutic agents to treat cryptosporidiosis. Additionally, *Cryptosporidium* oocysts are more resistant to chlorine disinfection and are smaller than *Giardia* cysts. Thus *Cryptosporidium* oocysts are more likely to pass through water treatment disinfection and filtration processes. Therefore, only the waterborne pathogen *Cryptosporidium parvum* will be considered here and in the current case study.

A.2.2.1 *Cryptosporidium* — *Cryptosporidium parvum* has been reported to be the major species responsible for clinical illness in humans (Current et al., 1986; Casemore, 1990; Rose, 1988) and in 79 different animal species (O'Donoghue, 1995). Discussions of *Cryptosporidium* resistance to chemical disinfection and other water treatment processes have been presented elsewhere in this document. Therefore the intent here is to present a brief overview of current information on *Cryptosporidium* and cryptosporidiosis in the general population and AIDS population.

A.2.2.2. *Cryptosporidium* Occurrence and Levels in the Environment — The levels of *Cryptosporidium* in surface water supplies have been reported by various authors; levels vary from region to region and appear to be dependent on environmental conditions and other contamination sources.

The average concentration of *Cryptosporidium* oocysts in surface source water used for potable water was determined for the case study and was based on the analysis of 60 quarterly samples and 12 monthly samples taken from raw water taps at the intake to the Trenton, NJ treatment facility. The samples were collected as part of an investigation conducted by

LeChevallier et al. (1998). Table A-2-2 *Cryptosporidium* Sample Analysis, summarizes the *Cryptosporidium* sample analysis compiled from the Trenton, NJ data.

A.2.2.3. Epidemiology — Many authors have published extensive and thorough epidemiologic reviews of *Cryptosporidium* (Soave and Armstrong, 1996; Fayer, 1997; Widmer et al., 1996; Juranek, 1997; Casemore, 1990a; O'Donoghue, 1995; Tzipori, 1988; Meinhardt et al 1996). *Cryptosporidium* was originally identified as a pathogen in livestock and not originally thought to be a human pathogen (Anderson and Bulgin, 1981; Anderson, 1982b; Barker and Carbonell, 1974; Angus et al., 1982; Tzipori et al., 1981). The first human cases of cryptosporidiosis were reported in 1976 (Meisel et al., 1976; Nime et al., 1976). In the United States, the first recognized waterborne outbreaks of cryptosporidiosis occurred in Braun Station (suburb of San Antonio), Texas in July 1984 and caused gastroenteritis in an estimated 117 individuals in 60 households. *Cryptosporidium* oocysts were found in the stools of 47 of 79 people who became ill (D'Antonnio et al., 1985).

The largest outbreak of waterborne cryptosporidiosis occurred during March-April 1993. An estimated 403,000 people who lived in and visited the 5-county area of greater Milwaukee, Wisconsin experienced a watery diarrheal disease due to *Cryptosporidium parvum* infection (MacKenzie et al., 1994). The spread of *Cryptosporidium* in water obtained from Lake Michigan was traced to one of two municipal water treatment facilities serving the area (Fox and Lytle, 1996). An advisory to boil water was issued on April 7, 1993 and the number of watery diarrheal cases dropped sharply after April 15, 1993. However, laboratory-confirmed cases continued to occur and a post-outbreak case control study was conducted. When people who had diarrhea during the outbreak were excluded from the analysis, immunosuppression was the only

TABLE A-2-2

Cryptosporidium Sample Analysis

No[1]	ID	Empty[2]	Amorphous[3]	1-4[4]	Total No[5]	per 100L[6]
1	1163	1	1	1	3	58.2
2	1164	0	0	0	0	<20
3	1165	0	0	0	0	<20
4	1166	3	1	1	5	100
5	1167	1	0	1	2	40
6	1168	0	1	0	1	20
7	1171	1	2	0	3	60
8	1172	2	0	0	2	40
9	1173	0	0	0	0	<20
10	1174	0	0	0	0	<20
11	1175	0	0	0	0	<20
12	1180	1	0	0	1	20
13	1181	0	0	1	1	20
14	1182	0	0	0	0	<20
15	1183	0	0	0	0	<20
16	1184	0	0	0	0	<20
17	1195	0	1	0	1	20
18	1212	0	0	0	0	<20
19	1225	0	0	0	0	20
20	1226	1	1	1	3	60
21	1227	6	5	3	14	280
22	1228	2	3	3	8	160
23	1229	0	1	0	1	20
24	1233	0	1	0	1	20
25	1234	0	0	0	0	<20

No[1]	ID	Empty[2]	Amorphous[3]	1-4[4]	Total No[5]	per 100L[6]
26	1235	1	0	0	1	20
27	1236	0	0	0	0	<20
28	1230	0	1	0	1	20
29	1237	0	1	0	1	20
30	1245	0	0	0	0	<20
31	1246	0	0	0	0	<20
32	1247	1	0	1	2	40
33	1248	1	0	0	1	20
34	1249	0	0	0	0	<20
35	1265	0	0	0	0	<20
36	1281	0	1	0	1	20
37	1295	0	0	0	0	<20
38	1296	0	0	0	0	<20
39	1297	0	0	0	0	<20
40	1298	0	0	0	0	<20
41	1299	0	1	0	1	20
42	1308	0	0	0	0	<20
43	1300	0	0	0	0	<20
44	1309	0	0	0	0	<20
45	1310	1	0	0	1	20
46	1311	3	3	1	7	140
47	1312	0	0	0	0	<20
48	1314	0	0	0	0	<20
49	1315	0	0	0	0	<20
50	1316	0	0	0	0	<20
51	1317	1	0	0	1	20
52	1318	0	0	0	0	<20

No[1]	ID	Empty[2]	Amorphous[3]	1-4[4]	Total No[5]	per 100L[6]
53	1326	0	0	0	0	<20
54	1336	0	0	2	2	40
55	1359	0	0	0	0	<20
56	1360	0	0	0	0	<20
57	1361	0	0	0	0	<20
58	1362	0	0	0	0	<20
59	1363	0	0	1	1	20
60	1374	0	0	0	0	<20
61	1375	0	0	0	0	<20
62	1376	0	0	1	1	20
63	1377	0	0	0	0	<20
64	1378	1	0	0	1	20
65	1388	0	0	0	0	<20
66	1379	0	0	0	0	<20
67	1389	0	0	0	0	<20
68	1390	0	0	0	0	<20
69	1391	0	0	0	0	<20
70	1392	0	0	0	0	<20
71	1411	0	0	0	0	<20
72	1433	1	0	0	1	20

[1] Number of samples
[2] Number of *Cryptosporidium* oocysts which were empty (Empty)
[3] Number of *Cryptosporidium* oocysts with amorphous internal morphology
[4] Number of *Cryptosporidium* oocysts which contained (1-4) of 4 possible sporozoites
[5] Total number of oocysts observed and
[6] The corresponding number per 100 L of sample.

risk factor associated with the post-outbreak illness. Some post-outbreak cases may have reoccurred in immunosuppressed people who were initially infected during the outbreak and there may also have been some person to person transmission (Osewe et al., 1996). Hoxie et al. (1997) reported that 54 cryptosporidial-related deaths among the residents of the Milwaukee area occurred within the 2 years following the waterborne outbreak. AIDS was the underlying cause of death in 46 (85%) of these.

More than 70 cases of cryptosporidiosis were reported during the Clark County, Nevada outbreak in 1994. Most of the cases (78%) were in immnuocompromised individuals. Tap water consumption was significantly associated with illness (odds ratio 4.22), although *Cryptosporidium* oocysts were not detected from drinking water sources during the study period. The municipal drinking water quality was reported to be better than that required by current federal standards (Goldstein et al., 1996), illustrating the resistance of this organism to traditional water treatment.

A.2.2.4 *Cryptosporidium* Prevalence Rates — Variation in prevalence rates may be due to geographic, demographic, temporal and methodological factors (Meinhardt et al., 1996; Casemore et al., 1985). Seasonal peaks have been identified in the United States in spring and late summer (Wolfson et al., 1985; Holley and Dover, 1986; Mann et al., 1986).

Non-outbreak prevalence rates of *Cryptosporidium* in screened stool specimens have ranged from 0.6-4.3% in North America and 1-2% in Europe. Prevalence rates in Asia, Australia, Africa, Central America and South America ranged from 3-20% (Fayer and Ungar, 1986; Current and Garcia, 1991; Sterling and Arrowood, 1993).

Surveys of U.S. populations have indicated that 15-50% of individuals are seropositive for *Cryptosporidium* antibodies (Ungar, 1990; Kuhls et al., 1994; Lengerich et al., 1993). The peak

incidence of infection appears to be in children between 1-5 years of age (Casemore, 1983, 1987; Palmer and Biffins, 1987). *Cryptosporidium* infections have been found in 4% of children with acute gastroenteritis and in 27-30% of asymptomatic children at day care centers (Unger, 1990). There may be a second peak incidence between 20 and 40 years of age, which could be due to familial contact with infected children. Clinical infection is less common after 40 years of age, and there is no evidence of elevated incidence in the elderly (Casemore, 1988).

A.2.2.5 *Cryptosporidium* Transmission Routes — *Cryptosporidium* infection can be transmitted through contaminated water or food, person-to-person contact, and contact with contaminated surfaces (Casemore, 1990b). Transmission from drinking water may go unrecognized (Juranek, 1995; Goldstein et al., 1996; Frost et al., 1996). Zoonotic transmission to humans who care for livestock (Angus et al., 1982; Tzipori et al., 1981) has been described, but transmission from livestock, zoo animals, experimental research animals, and companion animals may not be a major source of human cryptosporidial infection (Rose, 1988; Upton et al, 1989; Casemore, 1990b; Fayer, 1997; Blagburn and Current 1983).

A.2.2.6. *Cryptosporidium* Life Cycle — *Cryptosporidium* is an obligate intracellular parasite. An important part of the lifecycle is the excretion of mature and infective oocysts in the feces, which can then be passed directly or through sewage to terrestrial and aquatic environments (Gerba et al., 1995; Millard et al., 1994). Following ingestion by an appropriate host, the oocysts release several sporozoites that penetrate the epithelial cells of the gastrointestinal tract (Soave and Armstrong, 1986). These undergo several cycles of asexual reproduction, damaging the infected cells and infecting other cells. It is this asexual phase that causes most of the diarrheal illness associated with infection. Eventually, micro- and macrogametes are produced, which

combine sexually to produce new infectious oocysts that are released to the outside environment through the feces (Figure A.2-1) (Rose, 1988; Casemore, 1990b; Fayer, 1997). The oocysts are fairly resistant to environmental stressors (e.g., slow freezing, desiccation, physical abrasion, etc.) (Robertson et al., 1992) and to chemical disinfection (e.g., chlorine dioxide, chlorine, and monochloramine, etc.) (Korich et al., 1990; Peeters et al., 1989; Quinn and Betts, 1993; Parker et al., 1993; Liyanage et al., 1997; Parker and Smith, 1993).

A.2.2.7. *Cryptosporidium* Infectivity and Disease — The complex pathogenesis of the cryptosporidial diarrheal disease as well as the equally complex immune responses will not be discussed here, but have been reviewed elsewhere (Goodgame, 1996; Clark and Sears, 1996). The primary location of cryptosporidial infection is the distal bowel of the small intestine. Infection induces flattening of the intestinal villi, hyperplasia of the intestinal crypt cells and infiltration of inflammatory cells into the lamina propria (Clark and Sears, 1996). These morphologic changes interfere with enterocyte absorption and secretion (Goodgame, 1996; Griffin, 1990).

Studies indicate that the human infective *Cryptosporidium* dose may be in a range of 10-100 oocysts (Meinhardt et al., 1996; Fayer and Ungar, 1986). However, infectivity depends on the source, age and strain viability, as well as individual human susceptibility and immunity (Tzipori, 1983). *Cryptosporidium parvum* oocysts were fed to 29 healthy adults who had no serologic evidence of prior infection. The median infective dose (ID_{50}) for this particular Iowa

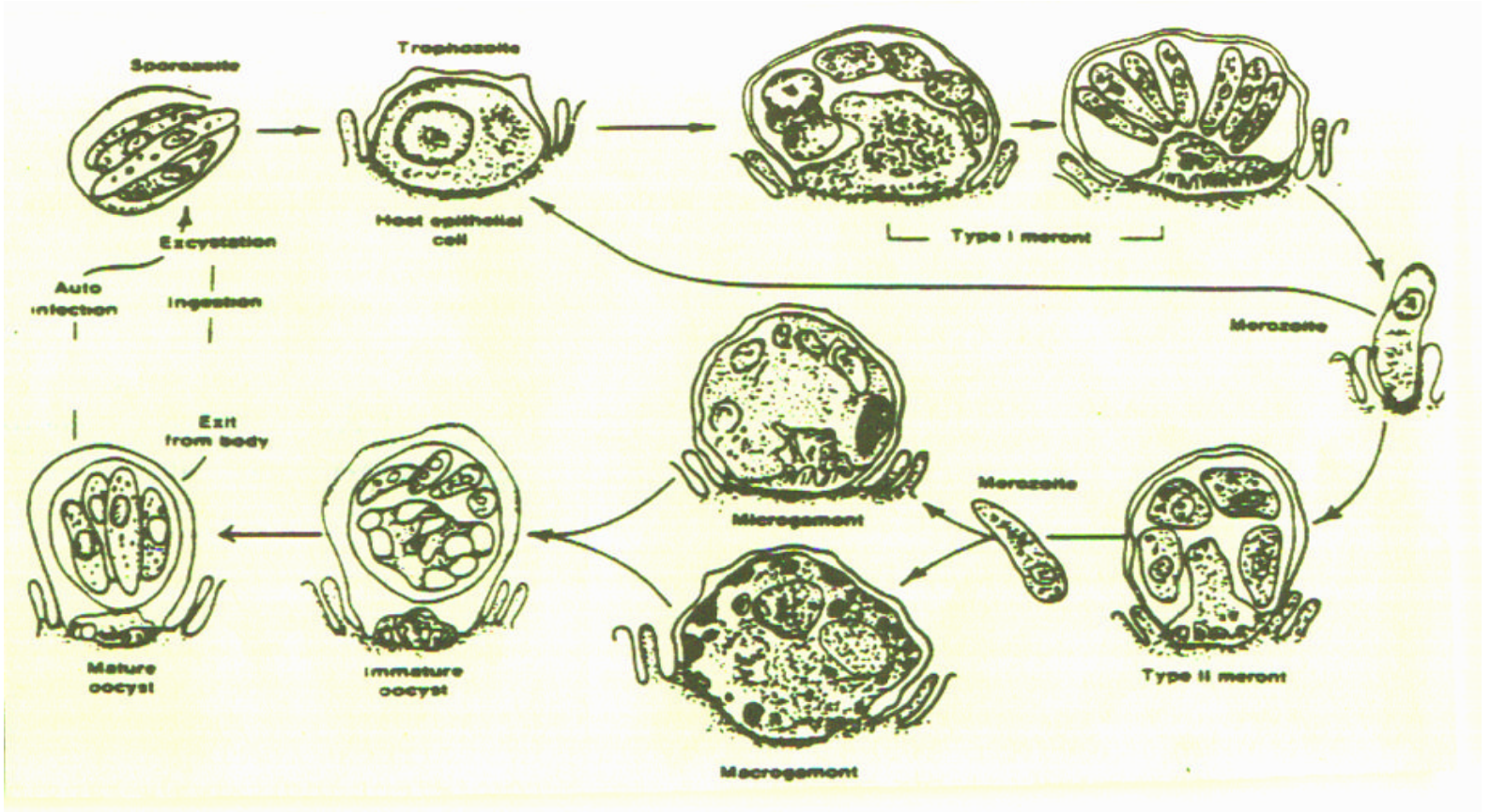

FIGURE A.2-1

Life Cycle of Cryptosporidium

Source: Fayer, 1997

strain was 132 oocysts and the minimum infective dose was less than 30 oocysts. The numbers of oocysts administered did not influence the incubation period (median of 6.5 days), the duration, or severity of the disease (Dupont et al., 1995).

A.2.2.8. Cryptosporidiosis in Immunocompetent Individuals — Typical symptoms of crystosporidial infection may include abdominal cramps, nausea, fever, vomiting, weight loss, watery and profuse diarrhea.. However, not all infections with *Cryptosporidium parvum* are clinically apparent (Dupont et al., 1995). The disease is usually self-limiting in immunocompetent people and symptoms typically resolve in 1-2 weeks. However, malnutrition or previously existing bacterial, viral or other protozoan infections may predispose individuals to more severe cryptosporiciosis (Ungar et al. 1988). Both humoral and cell mediated immunity are important for clearance of the disease, and immunoglobulins G and M (IgG and IgM) are detectable in the serum of infected individuals (Dupont et al., 1995). Roberts et al. (1985) reported that asymptomatic immunocompetent individuals may shed *Cryptosporidium* oocysts for 2 weeks.

The intensity of *Cryptosporidium* infection can be gauged by the number of oocysts found in the stool or a duodenal biopsy (Genta et al., 1993; Goodgame et al., 1993). Genta found that low-intensity infections were associated with normal duodenal morphology, and that severe inflammatory changes and villous atrophy were seen only in patients with high-intensity infections.

A.2.2.9. Cryptosporidiosis in Immunocompromised Individuals — The prevalence of infection is unknown but is estimated at 15% of people with AIDS (Greenberg and Koch, 1996). Immunocompromised people, including individuals with Human Immunodeficiency Virus (HIV) infection, may experience severe, prolonged, and life-threatening disease (Goodgame et al., 1993; Juranek, 1995; Goldstein et al., 1996; Greenberg and Koch, 1996; Colford et al., 1996).

However, immunocompromised patients with chronic symptomatic *Cryptosporidium* infections may routinely have negative stools specimens, and more specific diagnostic techniques have been recommended for these individuals (Greenberg and Koch, 1996). Baxby et al. (1985) found that 18% of 33 immunocompetent patients shed oocysts for less than 14 days, 40% for 15-21 days, 21% for 22-28 days and 21% shed for more than 28 days. The maximum duration of shedding in their study was 38 days.

In a recent cohort study of AIDS patients with *Cryptosporidium* infections, significant risk factors for death included a 5% weight loss within 3 months of diagnosis (risk ratio 2.53) or diagnosis of a prior opportunistic infection (risk ratio 3.34). Median survival was shortened from 16 to 7 months for AIDS patients who experienced a 5% weight loss within 3 months of diagnosis and from 16.5 to 6 months for patients with a prior opportunistic infection (Clark, 1997). HIV infected patients with $CD4^+$ cell counts above 180 cells mm^{-3} clear the infection in 4 weeks, whereas a large majority of patients with lower $CD4^+$ cell counts develop persistent disease (Flanigan et al., 1992).

Infections in immunocompromised individuals may occur in extraintestinal sites such as the eye, respiratory tract, gall bladder, bile and pancreatic ducts, lymph nodes, testicles, ovaries, uterus and vagina (Fayer et al., 1990; Fleta et al., 1995; Martins and Guerrant, 1995). Concurrent infections (i.e., coinfections, or overlapping sequential infections) of *Cryptosporidium* with other waterborne enteric pathogens have been reported (Meinhardt et al., 1996; Isaac-Renton et al., 1987; Casemore, 1986, 1987, 1990; Duke, 1996; Wolfson, 1985; Jokipii and Jokipii , 1985).

A.2.2.10. Pathogen Dose Response Models for U.S. Populations — The Beta-Poisson, lognormal, and exponential dose-response models have been used to estimate the probability of

infection from exposure to a single infective *Cryptosporidium* oocyte. The exponential model has been shown to reasonably fit the data for protozoa (Haas, 1996) and has been used in the current case study. Haas (1983) estimated the probability of infection as a function of *Cryptosporidium* oocyst intake using the exponential dose-response function. This model specifies that the probability of infection is $1 - e^{-rN}$, where N is the number of oocysts ingested, and r is the dose response function infectivity parameter. Details relating to the calculations of infection probabilities are explained in Chapter 6. This relatively simple model should be viewed as providing only a rough estimation of the risk of *Cryptosporidium* infection. This, and similar, mathematical models do not take into account individual immunity or susceptibility, secondary transmission dynamics, or other factors associated with infection and disease.

A.2.2.11. Alternative Source Assessment Models — The waterborne disease outbreaks presented in Table A.2-1 illustrate that a wide variety of types of U.S. water systems are subject to failures that allow human exposures to waterborne pathogens. These failures can result in outbreaks such as those reported in this table. Although transient mild to moderate waterborne illnesses are not reported and these rates are obviously unknown, these exposures to waterborne microorganisms may contribute to a significant amount of gastrointestinal illness.

The risk assessment of drinking water systems should include evaluations of water treatment technology, system function, and failure rates. There is clearly a need to identify, perhaps on a case by case basis, water treatment systems potentially at risk and the types of failures likely (including those brought about by environmental impacts such as floods and droughts). The case study presented does not evaluate the failure rate of the three water

treatment systems. Clearly these treatment systems could be subject to infrequent failures (e.g., operator error, failure of mechanical systems, environmental impacts, etc.).

The addition of an engineering risk analysis of U.S. drinking water treatment and distribution systems would improve future analyses of finished drinking water supplies that use the CRFM. Use of fault tree analysis and rare event/extreme outcome statistical models would provide better estimates of the temporal distributions of pathogens in finished drinking water. Linking improved source assessment models with a population dynamic transmission model for evaluation of secondary spread would provide a more accurate estimate of the magnitude of waterborne microbial exposures and pathogen risk from the operation of these systems.

A.3. DBPS: BACKGROUND, HAZARD IDENTIFICATION AND EXPOSURES

As stated above, different spectra of DBPs are produced from different treatment regimens and also can arise due to qualitative and quantitative differences in raw water. The DBPs arising from a given treatment option are found admixed in finished and distributed drinking water, therefore people are exposed to a somewhat complex mixture of chemicals, each with unique toxicological characteristics. The exposure to these chemicals, for the purpose of this document, is governed by water intake, which the Agency assumes is 2 liters per day. The economical limitations of complete removal of DBPs dictates that consumers of municipal drinking water are exposed to low levels of DBPs daily. General toxic effects have not been observed in research animals exposed to similar concentrations in drinking water (Bull et al., 1982; Kavlock et al., 1979). In contrast, epidemiologic studies of chlorinated drinking water exposures in people (see Section 5.7) suggest associations with bladder cancer and possibly other cancers (Cantor et al., 1985, 1997; Morris, 1992; McGeehin et al., 1993; King and Marrett, 1996; Freedman et al., 1997) and limited evidence of reproductive and developmental effects (Bove et al., 1995; Kramer et al., 1992; Swan et al., 1998; Waller et al., 1998). Although there are few studies available on defined mixtures of DBPs, evidence exists of dose-additivity for liver effects in mice exposed to mixtures of trihalomethanes (THMs) (Gennings et al., 1997) and of synergistic activity by mixtures of dichloroacetic acid (DCA) and trichloroacetic acid (TCA) for promotion of cancer (Pereira et al., 1997). The majority of the available DBP toxicity data consists of *in vivo* or *in vitro* studies. In single-chemical animal studies at high DBP dose levels there is evidence of carcinogenicity, reproductive effects, developmental effects, and other toxic effects, particularly in the kidney and liver (Bull and Kopfler, 1991; NTP, 1985, 1986, 1989; Smith et al., 1989).

Additionally, there is evidence of mutagenicity from exposure to extracts of finished drinking water in *in vitro* studies (Kool et al., 1981; Loper et al., 1978; Nestmann et al., 1982).

Research questions of interest, then, surround the issues of establishing, explaining and estimating any substantive human health risks from exposure to the low levels of DBPs found in drinking water. Because toxic effects are not observed in animal studies when the exposures are to low doses and because the epidemiologic data are inconsistent across studies with only relatively weak associations noted, the existence of human health risks is questionable. If it is assumed that the human health effects suggested in epidemiologic studies are true, then several hypotheses can be posed to explain the discrepancies between the epidemiologic results and the lack of effects in animals exposed to finished drinking water.

Such hypotheses include:

- there is an effect from the mixture of DBPs that is at least additive (if not synergistic) in nature, so that studies of low levels of individual DBPs are inadequate to explain any observed health effects;
- effects in humans are due to the chronic, repetitive insult from daily exposure;
- animal studies differ from human exposures in ways such as differences in physiology, biochemistry and anatomy that prevent them from demonstrating the same outcomes;
- other substances in drinking water or other human exposures not related to drinking water contribute to the effects that are suggested by epidemiologic studies such that investigations solely focused on DBPs will not corroborate the epidemiologic findings.

The toxicity of DBPs may be most appropriately investigated as a mixture not only because the mixture best represents the nature of the human exposure, but also because of the way DBPs are currently monitored. For example, both THMs and haloacetic acids (HAAs) are

measured for regulatory purposes as total sums of the mixture components. (A component is defined here as a single chemical within a mixture.) In response to the Safe Drinking Water Act Amendment of 1996, maximum allowable concentrations in finished drinking water were proposed for total THMs of 80 µg/L and total HAAs of 60 µg/L for Stage I of the rulemaking process. Proposed future concentrations for Stage II put limits of THMs at 40 µg/L and HAAs at 30 µg/L. It seems logical then to examine whether a mixture of THMs or HAAs is more or less toxic than only the single chemicals within these mixtures.

Many factors are involved in determining the mixture of substances that may be found in finished drinking water. These include: the type and characteristics of source water; type of disinfection used, e.g., chlorine, chloramine, chlorine dioxide, ozone; additional treatments such as enhanced coagulation, granular activated carbon, reverse osmosis filtration; range of pH levels; seasonal variation, etc. The initial focus for the water purveyor will be on the quality of incoming water. While the removal of microorganisms is a major concern, the choice of an effective treatment includes consideration of which DBPs will result. Raw water with high turbidity values requires more intensive filtration than does less turbid raw water. However, physical measures such as pH, organic content and turbidity do not always reflect microbiological contamination or health risk (Juranek and MacKenzie, 1998). The definition of new and more effective treatment options may prompt the emergence of DBPs not previously characterized or considered as problematic. Because individual DBPs are not randomly combined, DBP mixtures which are "representative" of different treatment scenarios should be considered as a single exposure.

The most common DBPs on which there are some concentration data include the THMs, HAAs, haloacetonitriles, haloketones, aldehydes, chloral hydrate, and chloropicrin, among others

(Jacangelo et al., 1989; Krasner et al., 1989; Lykins et al., 1994; Miltner et al., 1990). An important factor to be considered when comparing across treatment types is the bromide content of the source water. Ozone generally will not form THMs except when the source water contains high bromide (Miltner et al., 1990). To further complicate the exposure scenario, the composition of DBPs constantly changes within the distribution system, so that the human exposure varies with time and location. Although estimates of exposure at the tap for any individual are highly uncertain without direct sampling, distributions of exposures or ranges of dose levels for DBPs for given treatment scenarios can be estimated using real measurements and modeling procedures. These estimates can then offer a way to express the DBP exposure in terms that can be combined with dose-response data to quantify health risks and estimate the uncertainty in those risks.

A.4. ESTIMATING DBP DOSES IN HUMANS AND COMPARISON TO ANIMAL DOSES

Because the dose often determines the endpoint, the precise description of DBP doses is critical. The development of individual relationships between dose and the concentration/time profile of the toxicant at the target tissue provides much more information than dose alone. Human DBP doses can be estimated by multiplying the concentration of DBPs (in µg/L) found in drinking water (see Table A.4-1) by the assumed consumption rate of 2.0 liters per day for adults or 1.0 liter per day for children. To facilitate comparison with the expression of dose in laboratory animal studies (mg/kg/day), the amount of DBPs consumed (mg/day) is divided by kilogram body mass (70 kg for adults and 10 kg for children). For regulatory purposes the expected human dose (mg/kg/day) for DBPs is to a large degree driven by concentration in distributed drinking water, and is refined by correcting for volume consumed and body mass. The shape of the dose-response curve for a given toxic effect, the quantitative extrapolation of these data to the human, and the relative dose encountered by the human are factors which determine human health risk estimates. The dose to the human often differs quantitatively, as well as in pattern of exposure, from the dose encountered in studies with laboratory animals. Several animal and *in vitro* studies have identified specific consequences associated with exposure to doses of DBPs individually and in mixtures, but not all of these effects have been considered in the derivation of risk. By convention, the effect noted at the lowest dose represents the critical effect, and risk for this effect is then estimated in humans and used to set regulatory standards for human exposure.

One important consideration is that while test animals may be administered large doses of chemicals, humans are exposed to much lower doses and on a different dose schedule: daily

TABLE A.4-1

Occurrence of Several DBPs in Drinking Water

DBP	Range of Concentrations Found in Drinking Water (µg/L)	Mean Concentration Found in Drinking Water (µg/L)
Chloroform	0.7 – 540	26.4
Bromodichloromethane	1.9 – 183	9.1
Dibromochloromethane	0.4 – 280	5.7
Bromoform	0.1 – 2.7	0.5
Trichloroacetic acid	4 – 103	38
Dichloroacetic acid	12 - 79	47
Chloroacetic acid	1 – 16	NA
Bromochloroacetic acid	5 – 28.7	NA
Bromoacetic acid	<0.5 – 1.2	NA
Dibromoacetic acid	0.9 – 1.5	NA
Dichloroacetonitrile	1.9 – 24	2*
Trichloroacetonitrile	<0.01 – 0.3	NA
Bromochloroacetonitrile	<0.3 – 10	1*
Dibromoacetonitrile	“0 to several”	0.5*
1,1,1-Trichloropropanone	0.35 - 1.8	NA
Chloropicrin	0.2 – 1.5	0.8
Chloral hydrate	2 – 19	NA

Data are from several original sources, as cited in Bull and Kopfler (1991), and from Krasner et al. (1989) and Lykins et al. (1994) involving chlorine-sand-chlorine treatment train.

*Estimated
NA = data not available

doses to rodents may be administered via gavage boli in a vehicle while humans consume these DBPs without the complicating effects of vehicle and may spread their water consumption over the course of the day. The exposure of test animals to higher doses is predicated on the (in)ability to detect small incidences of toxicity, as might occur with much lower doses. Increasing the dose increases the likelihood of detecting toxicity, although the toxic consequences noted following the administration of high doses may not reflect the severity or magnitude of the toxic consequences (if any) following the exposure to lower doses. The identification of potential toxicities of concern, their incidence given a particular dose, and the mechanism by which they become manifest are often a direct function of dose, while the model chosen to extrapolate these effects below doses experimentally encountered, also impacts the estimation of human health risk.

Effects from exposure to high concentrations of DBPs may not be useful for consideration in estimating risk from DBP exposure (e.g., CNS depression from exposure to chloroform). The primary goal of the following sections is to guide the user in the determination of which human health effects might be considered in setting health-based goals and regulatory treatment options. Rodent toxicity studies usually involve doses and schedules which are not reflective of the manner in which humans are exposed to DBPs. The use of different assumptions about the extrapolation of effects to doses below the range of experimental doses influences how these effects may be considered relevant to the drinking water-exposed human.

A.4.1. Human Exposure and the Dose-Response Evaluation. A multitude of factors influence the dose-response relationship, many of which are controversial. The exposure to very low doses of known toxicants may not be sufficient to trigger critical molecular events, or the damage produced may be slight enough to be efficiently repaired. Alternatively, adverse effects may be

occurring but at rates that are not easily detected in animal, clinical or epidemiological studies. A lack of response following exposure to these low doses, together with deficits in knowledge about specific biochemical processes in the human, frequently accounts for much of the uncertainty in the estimation of human health risk for a given effect. Because humans are exposed to much lower doses than those encountered in laboratory animal studies, the comparison of the likelihood of effects over a broad range of doses (the dose-response curve) is necessary to predict effects potentially encountered in humans. Several assumptions are necessary to predict human risk to doses appreciably lower than those in the range of experimental doses. In the NOEL approach, the highest dose producing no toxicity (the No-Observed-Effect Level, NOEL) in laboratory animals is used for extrapolation. This dose is divided by uncertainty and/or modifying factors that address aspects such as duration of exposure, animal-to-human extrapolation, human interindividual variability, etc, to establish a Reference Dose (RfD) for human exposure (U.S. EPA, 1998a). Exposure of the human to the chemical below the RfD is not likely to produce adverse effects. Alternate approaches (e.g., the benchmark dose approach) use more data sets and/or more available data to establish and quantify the dose-response relationship. These approaches go beyond setting limits of exposure below which risk is not likely. They establish dose-response relationships through which a quantitative estimate of risk at a given dose (e.g., driven by DBP concentrations in drinking water) may be estimated. Under these approaches, extrapolations are made between the origin (zero dose-zero response) or background rate and the dose-response curve (or its confidence limit) at a given level of response (e.g., 10% response). By including uncertainty and modifying factors as in the NOEL approach, or body weight scaling, as has been done for carcinogens, an extrapolation to human response can be attained. The latter

type of approach provides more information by allowing for the establishment of the dose-response relationship, rather than a single point estimate of risk. The reliability of predictions below the observed range can be increased either by increasing the number of observations within the observed range (more doses), reducing the variability at any given dose level by increasing the sample size, or by extending the range of doses tested. The degree of conservatism may be influenced by modifying the point of departure (for extrapolation to the origin) and/or by changing the assumptions about the shape of the dose-response relationship between the point of departure and the origin. When data are not available to define the lowest end of the dose-response relationship (zero response, the NOEL), a linear extrapolation from the point of departure through the origin may used as the default approach.

Confidence about the risk estimated under alternate approaches can be increased through several measures. Once the effect of interest has been identified, structure-activity relationships for that effect as produced by related compounds, as shown by their respective dose-response relationships, may be used to extend the lower bound or to increase confidence (reduce variability) in the range of concentrations available. Further, the mechanism involved in toxicity may be one which does not fluctuate with dose. For instance, a chemical producing DNA damage through direct action on the DNA may reasonably be predicted to hold a constant dose-response relationship, while toxicities whose modes of action rely upon saturation of detoxication mechanisms (e.g., glutathione depletion) or mechanisms which are only expressed at high doses (e.g. cellular necrosis underlying carcinogenicity) may not be predicted to produce a linear dose-response relationship. When appropriate, and when there are data available, a precursor (marker) event may be monitored as an indicator of imminent toxicity. For example, events such as the

formation of DNA adducts may be considered as potential markers for tumorigenicity, even though frank tumors are not observed in experimental animals. When chemicals are identified as "tumorigens" in laboratory rodents and/or humans, concerted effort should be made to identify the underlying mechanism. Although the Risk Assessment Guidelines of 1986 did not specifically address mechanisms of tumorigenicity, the inclusion of the threshold dose concept is consistent with identifying tumor-producing compounds as promoters (as opposed to initiators, for which thresholds do not exist). The Proposed Cancer Guidelines (U.S. EPA, 1996a) include provisions for the use of specific mechanistic data to aid the interpretative assessment of other toxicological evidence of carcinogenicity. While chemicals identified as tumor initiators (e.g., those exhibiting direct DNA damage) may directly extrapolate to human carcinogens, those compounds identified as tumor promoters bear close scrutiny. Tumor promoters have a distinct threshold along the dose axis of the dose-response curve below which no effect is observed. The likelihood of the human encountering this extrapolated dose determines the likelihood that such tumor-promoting activity will be observed in humans. The adequacy of the cumulative dose measure ("Concentration times Time" approach) to predict toxic consequences should be assessed on a case by case basis and modified to fit the chemical under examination.

A.4.2. Patterns of Dose. The exposure of the human to DBPs in drinking water occurs daily, and may be dispersed more or less uniformly over the course of the day, or encountered as discrete episodes interspersed throughout the day, depending on the situation and workload. In contrast, one prominent characteristic of some laboratory studies is the administration of bolus doses of toxicant, whether given through oral gavage (effect of vehicle discussed elsewhere) or injected (i.e., intraperitoneally). These differences in the pattern of exposure may in some degree

modify the toxic outcome. For instance, the administration of high doses of chloroform produce hepatic necrosis, an effect that may promote the occurrence of hepatic tumors in animals exposed to high bolus doses of this agent. Lower doses which do not produce the necrotic effect seem to be without carcinogenic consequence, and in some instances (carbon tetrachloride) may actually protect against subsequent doses that would otherwise be toxic. While the administration of high doses of chemicals to laboratory animals is necessary to produce statistically detectable numbers of individuals displaying toxic effects during the animal's lifetime, the dose schedule producing toxic effects in rodents should be carefully compared with the exposure regimen most relevant in the human. Specifically, low doses of a given toxicant may be metabolically detoxicated while higher doses may saturate the detoxication and/or repair processes. Under these conditions, a high dose may be necessary to produce toxicity.

A.4.3. Relative Human Dose. The estimation of a human equivalent dose aids the plausibility of extrapolation of toxic events. The goal of laboratory toxicity studies is to refine human health risk estimation. The definition of toxic endpoint, establishment of the dose-response relationship and delineation of mechanism are use ful in the extrapolation of laboratory-derived data to the human. In addition, the recent additional attention given to childrens' risk may necessitate further evaluation of "lifetime" exposures, given the relatively recent advent of some processes. Because the oral route is the one primarily responsible for the internalization of DBPs found in drinking water, that route should be emphasized. Data on the bioavailability of DBPs is scarce and the default assumption is 100%. When data are available, specifically constructed toxicokinetic models may be used to refine the encountered dose/concentration to the more relevant terms of tissue dose. These models should be constructed for each species of interest and validated by

comparison to actual tissue time-course data for optimum impact. Further, data describing whether the parent compound or a metabolites is responsible for mediating the toxicity may provide a more scientifically-based estimation of toxicologically-active dose.

A.4.4. Species-Comparison of Dose. The default assumption is that the pharmacokinetic and metabolic patterns of a toxicant are quantitatively and qualitatively comparable across species (U.S. EPA, 1996a). Due to the marked differences in the bioconversion of several toxicants observed among humans and experimental species (e.g., sulfation in pigs, glucuronidation in cats, etc.), this may be one area where additional research efforts could provide great benefits on a chemical-by-chemical basis. For species-comparisons of doses, indicators such as blood levels of the toxicologically-active species or other biomarkers of exposure improve the comparison of dose. Any discussion of the delivered dose (which is modified from the administered dose by inclusion of specific marker indices) should also address available data or assumptions about the toxicodynamics of the target organ, both in the test species and the human. In summary, the most relevant exposures and dosing regimens are those of a drinking water exposure carried over the lifetime of the animal species.

A.4.5. Identification of Critical Effects. Once DBPs of interest have been identified, specific attention should be given to defining the critical effect: the adverse effect that is identified at the lowest dose tested and which will be used to drive the estimation of risk. Although one effect may subjectively be considered more detrimental than another, the shape of their dose response curves and the relative expression of the biochemical or molecular target in the human may modify their overall relevance. Effects not likely to be observed in the human should be given the least degree of attention, while the production of effects more likely to be observed in humans

bears more attention. The relative lack of knowledge about human developmental and neurologic functions dictate that most effects observed in these systems in test animals be considered directly applicable to the human by default. However, for alterations in systems which are more understood (e.g., carcinogenesis) the identification of the specific underlying mechanism and knowledge about the degree to which that mechanism is expressed in humans may modify consideration of the effect as critical, regardless of the shape of the dose-response curve.

Any effective review of the acute and long-term toxicities produced by the DBPs identified should lead to a complete definition of toxicities of interest. For chemicals for which multiple effects were monitored in groups of animals exposed to a range of doses, those effects which occurred at lower doses should be critically evaluated. The dose-response of the individual effects noted from separate studies should also be compared, as discussed later.

A.4.5.1 Carcinogenicity — Although some chemicals induce cancer in laboratory animals and cancer also occurs in humans, the understanding of chemical-specific mechanisms impact their likelihood in humans. The production of tumors in the kidneys of male rats by D-limonene (and several other chemicals) is dependent upon interaction with a specific protein (alpha-2 microglobulin), whose expression is severely limited in the kidneys of humans. Due to the high degree of certainty about the mechanism of toxicity for this chemical, and the relative lack of expression of the pertinent mechanism in humans, concern over the nephrocarcinogenic potential in humans is low. Another example of such an effect may involve the exposure to aniline dye components, where bladder tumors are expressed at exposure levels high enough to drive recrystalization of the compounds in the urinary bladder causing epithelial irritation as the

causative event. Because (presently regulated) human exposures are much lower, the concern for bladder tumors in exposed humans is low.

While the background incidence of liver tumors in rodents is relatively high, incidences for tumors at other sites (including the colon) are relatively rare. The production of a rare tumor type (angiosarcoma from vinyl chloride) or a tumor in a site rarely encountered (colon cancer from bromodichloromethane, BDCM) aids the detection or low incidences of a given response. In the case of colon cancer identified in laboratory animals exposed to BDCM, the effect may be especially relevant to humans.

A.4.5.2. Reproductive and Developmental Toxicity — As alluded to previously, almost any effect noted in the developing laboratory animal as a result of chemical exposure is treated as if it is directly applicable to the human, within the constraints of the Agency's Guidelines for Developmental Toxicity (U.S. EPA, 1996b). This decision framework considers "reproductive toxicity" to mean any effect that reduces reproductive success. This effect may be observed in dominant-lethal assays or in those which demonstrate significant alterations of sperm count and/or motility. Special consideration should be given to those compounds whose effect on sperm are reversible upon cessation of exposure. Reproductive effects in females may be indicated by altered estrus cycles, or hormonal imbalances normally associated with altered ovarian function. "Developmental toxicity" has previously been defined by the Agency (U.S. EPA, 1996b). Consistent with that definition, developmental toxicity, here, is defined as adverse effects upon the developing organism, and all effects identified as developmental in nature (structural malformations, embryolethality, alterations of neural function, fetal/neonatal weight gain, etc.) are weighted equally for the decision tool. Because the toxicities which result from

exposure during and prior to embroygenesis and the neonatal period may persist for a lifetime, they should be critically evaluated. Appreciable difficulty arises in distinguishing between the effects resulting from chlorine and those resulting from DBPs and is especially troubling when evaluating the effects of chlorine in human populations. Consideration of reproductive and developmental toxicity should relate the effect(s) noted with the specific causative chemical, when possible. Similar to the considerations of dose, the most relevant studies are those which employed the administration of the test chemical in drinking water. Guidelines established by the U.S. EPA for the assessment of developmental toxicity (U.S. EPA, 1996b) should be consulted to best facilitate the qualitative and quantitative interpretation of data reported for this endpoint.

A.4.6. Mechanisms of Toxicity. The demonstration of a dose level, below which toxic effects are not observed, expected or of concern, and above which effects are observed may indicate that some specific compensatory capacity or mechanism has been overcome. This effect should trigger additional investigations of the mechanism underlying toxicity. The evaluation of mechanistic data (when available) may determine the relevance to the human of the observed toxic insult. For instance, several classes of chemicals (e.g., herbicides, insecticides, etc.) produce discrete biochemical effects (plant hormone regulation, acetylcholinesterase inhibition, etc.) in non-human species that are not likely to be observed in humans for a number of reasons. When mechanistic data are available, they may be used to produce an estimate of the degree of interaction among DBPs. In instances where multiple chemicals are present, and which produce toxicity through the same biochemical insult, but are present at levels that may not be predicted to produce toxicity individually, their concentrations may be combined for a more accurate prediction of risk due to the specific biochemical insult. Non-biochemical mechanisms may also

determine the expression of toxicity and should not be overlooked. Another, less biochemically sophisticated example is provided by nitrilotriacetic acid (NTA), an additive in laundry detergents. NTA is generally non-genotoxic *in vitro* and *in vivo* but chelates calcium and zinc and produces urinary tract and bladder tumors in rats when exposed in feed. NTA is also absorbed 4-fold less in humans than rats. When the incidence of bladder tumors was compared to dose, it was shown that tumors in rats were found only at doses which increased urinary calcium. When regulators also considered that NTA was absorbed by humans only one-fourth as efficiently as in rodents, the compound was deemed not likely to pose a health threat to humans, and an exposure limit of 200 mg/L in drinking water was established. (See also urinary recrystalization of dyes in the section above.)

A.4.6.1. Pharmacokinetics and Metabolism — The absorption, distribution, metabolism and elimination of compounds often mediates which toxicities are observed, as well as potentially impacting the degree of severity for a given dose of a compound. Species-related differences in toxicity may be related to differences in parameters describing xenobiotic absorption, distribution, metabolism and elimination; although the bioavailability of orally administered compounds is generally 100%, their bioactivation or detoxication through metabolism may differ both among and within species. The incorporation of uncertainty factors of 10 have been employed to address variability among and within species, and incorporation of an additional modifying factor to protect children has been suggested. Metabolic capacity varies within and among species, and may either increase or decrease the toxicity of administered compounds. For compounds which must be metabolized (bioactivated) to produce a toxic response, a comparison of this potential in both the target species and the human should be

evaluated, and may be considered as a mechanistic modifier of toxicity. For compounds which are metabolized to less toxic forms (detoxicated), co-exposure to an enzyme inhibitor (as is common with pyrethroid insecticides) may increase toxicity.

A.4.6.2. Receptor-Mediated Effects — Like metabolic mechanisms, toxicity may be manifest from the kinetically-determined interaction between a toxicant and a biochemically-characterizable receptor. Several chemicals which bind and activate the same receptor with the same end result. On the other hand, two components of a mixture may bind the same receptor while one activates the receptor and the second inactivates it. In addition, other factors may modify binding efficiency. The affinities of individual chemicals for the receptor may indicate that much lower concentrations of one chemical may effectively out-compete (antagonize) the binding of the second chemical to the receptor, without respect to the consequence of binding.

A.4.6.3. Cancer-Related Mechanisms — The co-exposure to known tumorigenic compounds may also stimulate the assessment of the potential for interaction. For instance, the concurrent presence of tumor promoters and tumor initiators in drinking water might be predicted to produce a higher risk than the presence of either alone, given that the dose level of the promoter was sufficient to produce the promotional event. Likewise, the presence of two or more components in a mixture which adversely effect different steps in a cascaded pathway may produce an effect which is at least synergistic. An example of this might be the presence of a chemical which induces DNA adducts in *in vitro* assays and the presence of a second chemical which reduces the efficiency or efficacy of DNA repair mechanisms. Another example of this type of effect is illustrated by the increased level of DNA replication and hepatic histopathology produced by chloroform when administered in a corn oil vehicle versus the same dose when

administered in water (Pereira, 1994). Several factors intrinsic to corn oil including metabolic and pharmacokinetic effects may be causative. It should also be recognized that there are some instances in which the toxic effects of two chemicals are reduced when they are co-exposed. For instance, the mutagenic potential of chloroform *in vitro* is reduced when co-exposed with other mutagenic THMs, although the mechanism has not been determined.

A.4.7. Identifying DBPs of Greatest Concern. The goal of the present framework analysis approach is to disseminate a method by which a comparison of the health risks associated with drinking water disinfectants/disinfectant byproducts and the risks associated with infection/disease from microbiological contaminants in drinking water can be compared. The initial step must be to identify which agents (chemical or biological) may be found in drinking water. Several efforts have been directed at the delineation of DBPs and potential DBPs of concern. A rather lengthy list of over 700 chemicals has been compiled based on actual survey and laboratory production data (Richardson, 1998), while more than 500 DBPs have been identified in tap water (Clark et al., 1996). More refined lists which identify specific DBPs have compared their formation with the process treatment trains (e.g., the impact of pre-ozonation and chloramination on DBP formation relative to chlorination alone; Miltner et al., 1990). A formal listing of DBPs of primary concern has been published as the Contaminant Candidate List (CCL) (U.S. EPA, 1998).

Several DBPs have already been specifically identified as problematic due to the production of tumors in laboratory animals and the uncertainty about the mechanism and the dose-response relationship (e.g.,. dichloroacetic acid). The establishment of regulatory levels protective of public health have been mandated by Congress, and the Agency will follow suit on several more compounds soon. Deciding which treatment option to employ depends upon a

number of factors, including which DBPs may be reasonably anticipated to occur. The occurrence of these DBPs in drinking water is combined with data on water consumption (assumed for regulatory purposes to be 2.0 liters per day for adults) and provides the upper bound for the exposure estimate. By identifying the human health risk associated with critical (anticipated) DBPs, the treatment options for best minimizing risks can be determined. In the case of screening, the inclusion of the most conservative (worst case) assumptions might be included in the assessment. If a compound is identified as posing minimal risk under those considerations, it should be given a lower priority rating, but should be considered nonetheless. To facilitate this process, critical peer reviewed data for DBPs is being gleaned from the open and government literature and concentrations of DBPs in drinking water are being determined. Data describing both the toxicity and the distribution of these DBPs are being evaluated, and the chemicals are being considered for prioritization based on 1) occurrence, and 2) toxicity. Once listed, chemicals will be reevaluated for “occurrence” and “toxicity” data. Occurrence data for individual DBPs will be categorized by 1) the maximal reported concentration in any distribution system, 2) the number of utilities which have identified the DPB, and 3) the total population serviced by utilities which have identified the DBP. The presence of chemicals of interest will be determined by consulting reporting logs from treatment facilities and governmental surveys. From an occurrence standpoint, most community water systems supply less than 500 people each (63% of the total systems U.S.-wide supply water to only 2-3% of the population), while a few major systems (5.4% of total systems) supply water to over 75% of the population. It follows that focussing on the issues involving these few systems will impact the majority of the U.S. population. The available toxicity data for all chemicals initially given priority status will again be

evaluated for the type response identified in the species evaluated. When no data are available for the DBP, toxicity data for related compounds will be considered by employing structure-activity relationships (SAR). Applicable SAR models should be constructed based on knowledge or postulation of mechanism of toxicity and should be based on pertinent data such as applicable metabolites, route of exposure (focus on drinking water), tissue dosimetry, target organ toxicity, relative potency, etc. Available data will be sorted into cancer/non-cancer related toxicity and subjected to evaluation for quality and completeness of the data set. Once the toxicity data have been reviewed, priority ranking for DBPs will be reestablished by combining toxicity and exposure metrics. To this list will be added chemicals identified by mandate or for which new exposure guidelines were established since the initiation of the prioritization (Figures A-4-1 and A-4-2).

A.4.8. Individual Chemical Summaries. Summaries of toxicity and exposure information should be constructed for each DBP of interest. A partial list of sources of data can be obtained from the sections above, and other sources should be recruited as required. The summary list of toxic consequences which have been identified in laboratory animals should be culled to remove any whose mechanism diminishes their likelihood in the human. Pharmacokinetic data should be included as a basis upon which to modify exposure-related metrics between laboratory-exposed

animals and the human. Details of the pharmacokinetic data may include bioactivation and relative rates across species as modifiers of toxicity. Appendix A-5-4 gives chemical summaries for the DBPs of interest in the case study.

A.4.9. Research Needs. Specific consideration should be given to processes of distribution, absorption, metabolic activation/detoxication, biochemical mechanism of action, and the identification and quantitation of some of the more important factors that may influence interspecies extrapolation of rodent toxicity data. Specific research needs for DBPs of interest include additional data for *in vivo* dose-response of the effect of interest, identification of the minimally active dose-to-target-tissue producing the effect, data describing the mechanism of toxicity, and the determination of major factors which determine interspecies differences in toxicity. Additional research should be performed to define DNA repair capacity across species and evaluate the impact of such differences on the carcinogenicity of DBPs. The following series of questions are from areas where additional information-specific DBPs, DBP-associated events, and species-related modifiers of DBP toxicity will reduce some of the uncertainties in DBP risk assessment.

- Are THMs (genotoxic or epigenetic) carcinogens?
- Is metabolism necessary for TMH carcinogenicity?
- To what extent do free radicals play in THM carcinogenicity?, and
- Can sequestration of radicals eliminate THM toxicity?
- Like carbon tetrachloride, can low doses of THMs produce a cytoprotective effect?
- What term best describes the toxic interaction of THMs in a mixture?
- What tissue levels of active compounds are associated with toxic events?

- By what mechanism do halo-acetic acids produce tumors?
- To what extent might a metabolite produce the toxicity associated with halo-acetic acids?
- Can the tumor-producing activities of DCA be separated from peroxisomal proliferation?
- For metabolically activated DBPs, what is the relative degree of activation in humans?
- What are the relative rates of metabolism (activation or detoxication) in children?
- For genotoxic DBPs, what mechanism is responsible for repair of the lesion? and
- To what relative extent is this repair mechanism expressed in humans?
- What factors may modify the toxic response in children?

A.5. DBP RISK ESTIMATION USING RESPONSE-ADDITION

The U.S. EPA published the *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986) in which three approaches to quantitation of health risk for a chemical mixture are recommended, depending upon the type of available data (Teuschler and Hertzberg, 1995). In the first approach, toxicity data on the mixture of concern are available; the quantitative risk assessment is done directly from these preferred data. In the second approach, when data are not available for the mixture of concern, the Guidelines recommend using data on a "sufficiently similar" mixture. Similarity is judged from data on component composition of the mixture, component proportions within the mixture, source of emission, and health effects due to exposure to these similar mixtures. If the mixture of concern and the similar mixture are judged to be similar, then the quantitative risk assessment for the mixture of concern may be derived from health effects data on the similar mixture. Finally, the third approach is to evaluate the mixture through an analysis of its components, e.g., using dose-addition for systemic effects and response-addition for estimates of cancer risk. These procedures include a general assumption that interaction effects at low dose levels either do not occur at all or are small enough to be insignificant to the risk estimate.

The Guidelines recommend the incorporation of interactions data when available, if not as part of the quantitative process, then as a qualitative evaluation of the risk. There are many terms used to describe interaction effects among chemicals, but the two most common are synergism (the effect of the combination is greater than that suggested by the component toxic effects under additivity) and antagonism (the effect of the combination is less than that suggested by the component toxic effects under additivity) (Hertzberg et al., in press). Interaction effects are more

likely to occur at higher dose levels where toxicologic processes are affected, e.g., competition for receptor sites by a mixture of chemicals with a similar mechanism of action can result in an antagonistic effect.

As stated above, dose-addition and response-addition are similar in that they are both component based approaches that do not include interactions information because they are generally applied to mixtures that occur together at low dose levels. Under dose-addition, the first step is to scale the doses of the components for potency and add the doses together; the mixtures risk is then estimated for the combined mixtures dose. Under response-addition, the risks are first determined for the individual components; the mixtures risk is then estimated by adding the individual risks together. These processes are fundamentally different and require different assumptions of the data in order for them to be used appropriately.

Dose-addition is different from response-addition because two assumptions are made: that all of the components have similar uptake, pharmacokinetics and toxicologic processes, and that the (log probit) dose-response curves of the components are parallel (Teuschler and Hertzberg, 1995). This means that, for equal effects, the dose of one component is a constant multiple of the dose of a second component. Usually the assumption is made that the same constant multiple applies to any effect. Hertzberg et al. (In press) note that dose-addition often does a reasonable job of predicting the toxicities of mixtures composed of a variety of both similar and dissimilar compounds (Pozzani et al., 1959; Smyth et al., 1969, 1970; Murphy, 1980; Ikeda, 1988; Feron et al., 1995), although exceptions have been noted. Dose-addition is particularly useful in situations where the dose for each individual component is at a level at which effects are not expected to occur or be observable; when the doses are combined, effects are then expected or observed in

response to the higher dose level of the mixture. Often, dose-addition is applied by scaling the potencies of all the components in the mixture to an index chemical, adding the scaled doses together to give the equivalent dose in terms of the index chemical, and using the index chemical's dose-response curve to estimate risk for the total mixture dose.

Response-addition is different from dose-addition in that it does not assume similar kinetics or a similar mode of action and does not assume parallel dose-response curves. It assumes that the components of the mixture are considered to be functionally independent of one another at low exposure levels (Mumtaz and Hertzberg, 1993), so that the risks may be added together. Because response-addition does not require a similar mode of action across the chemicals in the mixture, it allows for combining risks across different types of endpoints, unlike dose-addition. Response-addition is particularly useful when the effect of concern is thought to be present at low dose levels for each of the component chemicals, even though it is highly unlikely to be observable at these low levels in either epidemiologic or toxicologic studies; the mixture risk is then the sum of the individually low risks of the independently acting component chemicals. For example, response-addition has often been used for the risk assessment of mixtures of carcinogens (Gaylor et al., 1997; U.S. EPA, 1989).

The approach that is to be applied within the comparative risk decision analysis is to work with component information, using appropriate additivity assumptions to combine data on exposure levels for specific drinking water treatment scenarios with dose-response estimates from animal data to generate human health risk estimates for the mixtures of concern. The advantage of using the single chemical dose-response toxicity data and combining them with treatment-specific exposure information is that this method allows for relative comparisons of health risks across the

treatments. In addition, it allows for future health risk comparisons of any proposed decreases in the allowable levels of DBPs in the finished drinking water. Any available interactions data indicating that the mixture components may interact in a synergistic or antagonistic way will be used only as qualitative information in the analysis.

Although estimates of human cancer, reproductive or development risks may be taken from the epidemiologic literature, these data do not distinguish the risks across various treatment scenarios. Most of the epidemiologic data simply distinguishes between chlorinated or non-chlorinated water, or between chlorinated surface water and groundwater without a detailed exposure characterization (Morris, 1992). Therefore, dose-response estimates of health risks across treatments cannot be easily obtained. Additional data from epidemiologic studies may be used from the perspective of generating data used in establishing the human dose-response relationship, as a guidepost indicating which biochemical mechanism to evaluate for potential interaction with other chemicals, to corroborate estimates of human health risk calculated from the animal data, or to provide upper bound risk estimates as part of the sensitivity analysis. Human exposures are difficult to interpret, owing to exposure to a multitude of chemicals daily. Some epidemiologic studies have been criticized for this, but those studies still present data which may be useful, if only qualitative. Studies correlating observed toxicity with exposure to agent(s), whether from laboratory animals or humans, are of more value when specific components are identified. Some investigations have been performed on human populations where the only identifier is “disinfection with chlorine” versus “disinfection with chlorine dioxide”. While those data are better than none at all, a more clearly defined exposure regimen would be beneficial.

This is rarely the case in more well controlled studies with laboratory animals, where exact doses of DBP chemicals are known.

A.5.1. Pilot-Scale Investigation of the Formation and Control of Disinfection Byproducts.

The paper by Miltner et al. has been incorporated in Appendix A.5.1.

A.5.2. Quantitative-Structure-Toxicity-Relationship (QSTR) Report on DBPs.

A.5.2.1. Background — The earliest version of TOPKAT® (Toxicity Prediction by Komputer-assisted Technology) was introduced in 1987 by Health Designs, Inc. A complete redevelopment of the earliest version led to the introduction of TOPKAT 3.0 in 1995. Subsequently, TOPKAT 3.0 was enhanced to 5.0 in 1997 to include support for moiety analysis. These software packages are designed for the accurate and rapid assessment of the toxicity of chemicals solely from their molecular structure. Both software packages utilize robust, cross-validated Quantitative Structure-Toxicity Relationship (QSTR) models for assessing specific health- related endpoints, e.g. rodent oral carcinogenicity (NTP), Ames mutagenicity, developmental toxicity potential, rat oral chronic Lowest-Observed-Adverse-Effect Levels (LOAEL), rat oral LD_{50}, skin sensitization, fathead minnow LC_{50}, and *Daphnia magna* EC_{50}. In addition, a Log P QSAR model is also available, where P is the ratio of the solubility in octanol compared to the solubility in water and is known as the octanol-water partition coefficient.

A.5.2.2. Structure-Based Models — Structure-based toxicity assessment approaches can be classified into two types; namely, Expert Systems (Human and Artificial) and QSTR approaches. Expert Systems approaches are based on a collection of rules derived from existing subject knowledge and stored in computer memory. QSTR-based model is a quantitative relationship between numerical measure of toxicity and a set of structural descriptors. The backbone of this approach is the effective quantification of salient structure attributes. Such attributes include electronic (valence, sigma, pi and lone-pair), bulk (molecular weight, size corrected E-values) and shape (molecular shape and symmetry) (Gombar, 1998). Such models are developed for predicting various endpoints (e.g.carcinogenicity and developmental toxicity)

and are based on very carefully selected studies from the literature using extremely stringent criteria.

A.5.2.3. Structure-Based Model Development — In principle, a structure-based toxicity model is a quantitative relationship between a numerical measure of toxicity and a set of structure descriptors, i.e.,

$$T = f(S)$$

where T is a measure of toxicity (e.g. LD_{50} , LOAEL, indicator of carcinogenicity), S is a set of numerical quantities representing different structural attributes, and f is a mathematical function (HDI, 19**). These structure-toxicity relationships are generally called quantitative structure-toxicity relationships (QSTR) models or equations, because by knowing the function, f, and providing the values of S for any chemical one could estimate its toxicity, T.

A special case in which (f) represents a linear multiple discriminant function is the QSTR equation below which is a algebraic summation of all identified descriptors to compute a probable value of toxicity for a submitted chemical structure. The form of a QSTR is:

Computed Toxicity Value (Discriminant score)= $(Coeff_1) \times (Var_1)$
+ $(Coeff_2) \times (Var_2)$
+ $(Coeff_3) \times (Var_3)$
+ .....
+ $(Coeff_N) \times (Var_N)$
+ Constant

The variables in the above QSTR equation are the calculated values of the structure descriptors; the coefficients are the statistical weights associated with these descriptors. During the development of the equation these weights are optimized. The product of the descriptor variable and the coefficient is the descriptor's contribution to the estimated toxicity. If the contribution is

positive it increases the probability of toxicity whereas a decrease in probability is denoted by a negative value. The descriptors used in TOPKAT 3.0/5.0 models quantify the electronic, shape and symmetry attributes of a molecular structure. The electronic attributes are expressed in terms of the electrotopological E-state values (Gombar, 1998) of specially designed 1-atom and 2-atom fragments of non-hydrogen atoms in different hybrid and hybridization states called HDi substructures.

Toxicity values are computed by summing the contributions of the individual descriptors. For assessing toxicity values such as the LOAEL and LC_{50}, this sum is transformed into a weight/weight unit (mg/kg) and weight/volume unit (mg/L). For carcinogens, mutagens, and developmental toxicants, this sum is transformed into a probability value between 0.0 and 1.0. For such 2-group classifications, a value between 0.0 and 0.3 is considered negative or of low probability; a value between 0.3 and 0.7 is considered indeterminate (i.e., too near equal probability) for an assessment to be meaningful and a value above 0.7 is considered to be positive.

A.5.2.4. Model Validation — These models are based on both discriminant and regression analyses using dichotomous and continuous scales, respectively. It is relatively easy to develop a tentative QSTR with good correlation (r^2); however, a good r^2 does not necessarily indicate that the model is appropriate for predictive purposes. Therefore, it is essential to further validate these models based on a variety of diagnostics (Gombar et al., 1997). These diagnostics include: (a) all descriptors in the function are significant, (b) no compounds with unique variables are in the training set, (c) no influential or outlier compounds remain in the training set, (d) residuals are normally distributed and (e) cross validation performance (r^2_{cv}) is not significantly

different from the performance (r^2) on the training set. Unless these characteristics are established in a QSTR, it cannot be considered robust and, therefore, its statistical quality is questionable.

A.5.2.5. Model Accuracy — In the case of the Developmental Toxicity Model (Gombar et al., 1995) stringent criteria was applied to 5559 open literature citations containing experimental data on developmental toxicity with the selection of 1238 rat studies for the development of the model. However, 830 of these bioassays were not usable due to inherent problems in protocols and 34 bioassays were deleted due to uncertain structure, organometallics and mixtures. Ultimately, the compounds suitable for QSTR models were reduced from 374 to 273 based on the fact that some studies were performed at only one dose only and both DT and MT were observed at that dose, and for some studies, neither DT nor MTD was reported even at the highest dose. Based on specific criteria a DTP score between 1 and 4 was assigned to each of the 273 chemicals. Ultimately, 273 chemicals were used in the development of three developmental toxicity submodels (aliphatic, carboaromatic and heteroaromatic). The cross-validation (leave-one-out) accuracy of the three submodels range from 86.1% to 88.6% in terms of sensitivity (known developmental toxicants identified as positive) and 86% to 97.4% in terms of specificity (known non-developmental toxicants identified as negative). The indeterminants ranged from 2.2 to 2.5%. Each submodel is comprised of about the same number of compounds to give a total of 273 compounds.

The NTP Rodent Carcinogenicity Model comprises four statistically significant and cross-validated QSTR models, and the data from which these models were derived. Each QSTR model relates to a specific sex/species combination: female rat, male rat, and female mouse, male mouse. The basis for each model are the 366 rodent carcinogenicity studies conducted by the National

Cancer Institute (NCI) and the National Toxicology Program (NTP) utilizing inbred rats and hybrid mice. In selecting the most appropriate studies, stringent criteria was uniformly applied to all NCI/ NTP studies and any studies not conforming to the predefined standards of purity, exposure duration, route of exposure, dose levels, etc., were not included in the training sets for developing QSTR models for carcinogenicity. For instance, results from 158 carcinogenicity assays in the female rat could not be included in the training set for various reasons (Gombar et al., 1997). With respect to the accuracies of these four carcinogenicity submodels, the sensitivity (% of known carcinogens predicted as carcinogens) ranged from 82% in the case of the male rat to 91% in the case of the female rat. Prediction of non carcinogens as non carcinogens (specificity) ranged from 82% in the case of the male rat to 94% for the male mouse. Percent indeterminants were 11, 1, 1 and 5 for the male rat, female rat, male mouse and female mouse, respectively. There were approximately equal number of compounds in each submodel to give a total of 815 compounds.

A.5.2.6. Quality Assurance/Control — An established data base was used to develop the QSTR models for predicting the various health-related endpoints of novel structures have been accumulated, evaluated and standardized the software developer. These data include, but are not limited to, chemical structure depictions, Chemical Abstract Service (CAS) Registry numbers, experimental toxicity values, and reference citations. All of the models in the software package have been developed by qualified statisticians, toxicologists, computational chemists, and computer programmers, specializing in QSTR.

A.5.2.7. QSTR Analyses —

Developing the hypothesis (prediction) via univariate and multivariate analyses

In general, QSTR models are limited in their applicability, since they are derived from experimentally measured toxicity values involving a limited number of descriptors. Consequently, it is important to determine whether the structural attributes (descriptors) of the query compound are represented in the compounds used for model development. In order to assess whether the estimated toxicity is meaningful or not, and to assure reproducibility of all the results, this software program has incorporated algorithms that have been thoroughly tested. These algorithms[2] are termed univariate (Coverage Examination) and multivariate [Optimum Prediction Space Examination(OPS)] analyses.

The univariate analysis determines whether all of the structural fragments of the query structure are well-represented in the model data base or training set and the multivariate analysis determines whether the query structure fits within or near the periphery of the OPS of the equation. The OPS of a QSTR is a multi-dimensional space, the number of dimensions being one more than the number of model descriptors of the QSTR. An important characteristic of the OPS is that within and near its periphery (permissible limits) the QSTR may be applied with confidence. If either of these criteria (univariate and multivariate analyses) are not satisfied, a warning is displayed; however, if they are satisfied "All Validation Criteria Satisfied" is displayed.

Testing the hypothesis via similarity search

Using "Similarity Search"[2] this hypothesis can be tested against compounds in the data base based on their QSTR similarity to the query structure. TOPKAT displays the actual experimental and predicted results, whether the compound was used in the training set, and the

similarity distance (normalized to the query compound) from the query on a scale of 0.0 to 1.0. The smaller the distance, the greater the similarity. Acceptable results are obtained if one finds that the experimental and predicted values of the "similarity search" compounds are in agreement and the normalized similarity distance is 0.35 or less for discriminant analysis and less than 0.2 for regression analysis. If the experimental and predicted values do not agree then the "similarity search" is considered unacceptable.

A.5.2.8. Quantitative Structure Toxicity Relationship Results — A total of two hundred fifty three compounds, identified as disinfection by-products (DBPs's) by the Office of Water, were analyzed for carcinogenicity and developmental toxicity using TOPKAT®/QSTR software. All submitted query structures for either endpoint were automatically subjected to univariate (Coverage) and multivariate (Optimum Prediction Space) analyses, and ultimately generated a "hypothesis" in terms of probability. Subsequently, the confidence in the hypotheses were tested by application of the "similarity search" algorithm. The application of such diagnostic tools within the software ensures the accuracy for each model prediction in terms of false-positives and false-negatives.

The initial agreement between NCEA-Cin and the Office of Water (OW) was to subject the 252 DBPs submitted by OW to QSTR analyses using TOPKAT software. In view of the time constraints it was agreed upon that such prioritization of DBPs would not include any rationalization as to why certain classes of chemicals based on functional groups are active and others inactive. In general, there are two reasons for prioritization; namely, regulatory decision making and research needs. This effort focuses principally on the research needs for Stage 2

DBPS Rule for which there is little health data available. Such data will assist OW in selecting high priority DBPs or classes of DBPs for additional health effects studies and eliminating others.

The results of the 253 DBPS's analyzed were tabulated and prediction patterns were observed within and between classes based on their functional groups (e.g. alcohols, acids, etc.) and specific health related end-points. A visual representation of the QSAR analyses for the principal functional groups are presented as bar graphs. An examination of the results obtained should aid in the prioritization of these health-related endpoints for performing additional bioassay studies.

Correlation of Carcinogenicity and Developmental Toxicity with DBPs.

Aldehydes:

Carcinogenicity:

The carcinogenicity of aliphatic aldehydes is predicted to be gender specific in that the majority aliphatic aldehydes (22/28) were predicted as carcinogens in the female mouse and non-carcinogens in the male mouse. All aliphatic aldehydes were predicted as non-carcinogens in female rats with a mixture of carcinogens/non-carcinogens (5/28) in male rats. The two aromatic aldehydes, benzaldehyde and benzacetaldehyde are predicted as non-carcinogens in all submodels with the exception of the female mouse which predicts benzaldehyde to be carcinogenic.

Developmental Toxicity:

With the exceptions of cyanoformaldehyde, tribromoacetaldehyde, methyl propanal and methyl glyoxal, all other aliphatic and aromatic aldehydes (20/30) are predicted negative for developmental toxicity.

Mono Carboxylic Acids:

Halogenated acetic acids:

Carcinogenicity-

All monohalogenated acetic acids are predicted as non-carcinogens for both female and male rats and mice with the exception of bromoacetic acid which is predicted as a carcinogen in female rats and mice. Dihalogenated acetic acids are predicted as non-carcinogens for all submodels with the exception of dichloro- and dibromoacetic acids which are predicted as carcinogenic in male mice. With the exception of trichloroacetic acid in the female rat, all other trihalogenated acetic acids are predicted as carcinogens in male and female rats and male mice. However, in the case of the female mouse submodel all trihalogenated acetic acids were predicted as non-carcinogens.

Dichloro-, dibromo- and bromochloroacetic acids are predicted as non-carcinogens and carcinogens in male rats and mice submodels, respectively. In female mice submodel, bromochloroacetic acid is predicted as a carcinogen.

Trifluoro-, tribromo- , dibromochloro- and dichlorobromoacetic acids are predicted as carcinogens and non-carcinogens in female rats and mice submodels, respectively.

Developmental Toxicity-

In contrast to carcinogenicity, all three classes of the halogenated acetic acids (mono-, di- and tri-), including mixed di- and trihalogenated acetic acids, show a distinct pattern. All the mono- and trihalogenated acetic acids of fluorine, chlorine, bromine and iodine, are predicted as developmental toxicants; whereas, all the dihalogenated acetic acids for the same halogens are predicted negative for developmental toxicity.

Chlorinated carboxylic acids (C3,C4,C5):

Carcinogenicity-

Due to the limited quantity of C3 to C5 chlorinated carboxylic acids, analyses failed to produce an overall pattern with respect to carcinogenicity, although 3/7 acids were predicted positive in more than one rodent model.

Developmental Toxicity-

All chlorinated carboxylic acids are predicted as developmental toxicants, with the exception of those that were judged to be indeterminant (2-Chloropropanoic acid, and 3,3-Dichloropropenoic acid) or outside the Optimum Prediction Space (OPS) [(2,3-Dichloro-4-oxobutenoic acid)].

Non-halogenated Carboxylic acids:

Carcinogenicity-

The majority (21/27) of non-halogenated carboxylic acids were predicted as non-carcinogens for all four submodels (male/female rats and mice). The exceptions were glyoxylic acid and 1,2-dioxopropanoic acid, which were predicted as carcinogens in female mice and rats, 2-oxopropanoic acid, 2-methyl propanoic acid, dioxobutanoic acid, 4-oxopentanoic acid which

were predicted as carcinogens in female rats submodel, and decanoic acid, undecanoic acid and dodecanoic acid which were predicted as carcinogens in male rats submodel. Many of these compounds were judged to be outside the Optimum Prediction Space (OPS) in the male mouse and rat submodels.

Developmental Toxicity-

Non-halogenated carboxylic acids ranging in carbon chain lengths from C2 to C5 are predicted as developmental toxicants, with the exception of 2-oxopentanoic acid, which is predicted negative. Acids with carbon chain lengths greater than C6 are all predicted negative, with several of these compounds judged to be outside the Optimum Prediction Space (OPS).

Di Carboxylic Acids:

Chlorinated Di-Carboxylic Acids:

Carcinogenicity-

As in the case of chlorinated mono-carboxylic acids, there were not enough chlorinated di-carboxylic acids analyzed to see an overall distinct pattern in prediction.

Developmental Toxicity-

Of the three compounds analyzed, 2-chlorobutenedioc acid is predicted as a developmental toxicant, chlorobutanedioic acid was predicted as a non-developmental toxicant and 2-chloro-3-methyl-cis-butenedioic acid was predicted as indeterminant.

Non-halogenated Di-Carboxylic Acids:

Carcinogenicity-

The majority (14/16) of non-halogenated di-carboxylic acids were gender and species specific for carcinogenicity, with carcinogenicity limited to the female rat sub-model. These acids

were predicted non-carcinogens in male rats and mice and female mice sub-models. Tert-butyl-cis-butenedioic acid and tridecanedioic acid are exceptions, being predicted as carcinogenic in female mice and non-carcinogenic in female rats sub-models, respectively.

Developmental Toxicity-

Most non-halogenated di-carboxylic acids (12/16) are predicted as developmental-toxicants, except octanedioic acid, nonanedioic acid and tridecanedioic acid, which are predicted negative and heptanedioic acid which is indeterminate.

Aromatic Carboxylic Acids:

Carcinogenicity-

Aromatic carboxylic acids (7/12) are predicted as carcinogens in the female rat submodel and non-carcinogens in the male mouse and rat and female mouse submodels. Three acids (1,2,3-, 1,2,4- and 1,3,5-benzene tricarboxylic acids) are judged to be outside the Optimum Prediction Space (OPS) in male mice and female rats submodels.

Developmental Toxicity-

All aromatic carboxylic acids are predicted negative for developmental toxicity, with the exception of phenylacetic acid which is predicted positive.

Ketones:

Halogenated Ketones:

Carcinogenicity-

Most halogenated ketones are predicted as carcinogens in the male mouse sub-model and predicted as non-carcinogens in the female mouse and rat and male rat sub-models with the exception of 1-chlorodimethylglyoxal, chloropropanone, 1,1-dichloropropanone, 1-bromo-1,1-

dichloropropanone, 1,1-dichloro-2-butanone and 3,3-dichloro-2-butanone which are predicted as non-carcinogens in the male mouse sub-model. 1,1,1,3-, 1,1,3,3-tetrachloropropanone and hexachloropropanone are predicted positive for carcinogenicity in the male rat and 1,1-bromochloropropanone is predicted positive in female mouse and male mouse. Approximately 50% of these ketones (19/40) were determined to be outside the Optimum Prediction Space (OPS).

Developmental Toxicity-

Except for 1,1-dibromoacetone, 1,1-dichloropropanone, 1,1,3-trichloropropanone, 1,1,1,3,3-pentachloropropanone, 1,1-bromochloropropanone, and 1,1-dichloro-2-butanone all halogenated ketones are predicted positive for developmental toxicity.

Non-Halogenated Ketones:

Carcinogenicity-

Of the eleven non-halogenated ketones, most appear to be gender and species specific for carcinogenicity, in that , they are predicted carcinogenic in the male rat sub-model with a few exceptions. These exceptions being, butanone, 3-hexanone and, 6-methyl-5-hepten-3-one which are predicted non-carcinogens. Glyoxal is predicted positive in the female mouse as well as the male rat sub-model. Three compounds are determined to be outside the Optimum Prediction Space (OPS) for all four sub-models.

Developmental Toxicity-

Most non-halogenated ketones show a positive trend for developmental toxicity. Geranylacetone and 3-methyl-2,4-hexanedione are judged to be outside the OPS and 3-hexanone, 2,6-Dimethyl-2,5-heptadiene-4-one and 6-methyl-5-hepten-3-one are predicted negative.

Cyclic Ketones:

Carcinogenicity-

All cyclic ketones appear to follow a non-carcinogenic trend in all four sub-models, with only 2,2,4-trichloro-1,3-cyclopentenedione being predicted positive in the female mouse. 2-chlorocyclohexanone is determined to be outside the OPS in the male mouse and 2,6,6-trimethyl-2-cyclohexene-1,4-dione and 1,3,3-trimethyl-1,7-oxabicyclo-[4.1.0]-heptane-2,5-dione are indeterminant.

Developmental Toxicity-

Of the seven cyclic ketones, four are judged to be outside the OPS, whereas 2,2,4-trichloro-1,3-cyclopentenedione, 2-chlorocyclohexanone and 1,3,3-trimethyl-1,7-oxabicyclo-[4.1.0]-heptane-2,5-dione are predicted negative for developmental toxicity.

Aromatic Ketones:

Carcinogenicity-

There is no clear pattern evident for carcinogenicity in aromatic ketones. 1-[4-(1-methylethyl)phenyl]-ethanone and 1,1-(1,4-phenylene)bis-ethanone are predicted positive in the male mouse and rat sub-models, whereas 2,6-tert-butyl-1,4-benzoquinone is predicted positive in the female mouse sub-model and is indeterminant in the male rat sub-model. The remaining aromatic ketones are predicted as non-carcinogens.

Developmental Toxicity-

All aromatic ketones are predicted negative for developmental toxicity.

Lactones:

Carcinogenicity-

The lactones appear to have their carcinogenicity restrained to the male mouse sub-model. Apart from dihydro-4,5-dichloro-2-(3H)-furanone, red-MX, EMX and 4-dodecyl-5-ethyl-2(5H)furanone which are predicted non-carcinogens in the male mouse and MX which is predicted indeterminant in the male mouse all other lactones are predicted as carcinogens in the male mouse sub-model. 5-Hydroxy-5-trichloromethyl-2-furanone, BMX-1 and EMX are predicted as carcinogens in the female mouse sub-model. Many of the lactones are judged to be outside the OPS, especially in the female rat.

Developmental Toxicity-

Most lactones are predicted negative for developmental toxicity. The few exceptions being, 5-hydroxy-5-trichloromethyl-2-furanone which is predicted indeterminant and dihydro-4,5-dichloro-2-(3H)-furanone, red-MX and 4-dodecyl-5-ethyl-2(5H)furanone which are judged to be outside the OPS.

Alcohols:

Aliphatic Alcohols:

Carcinogenicity-

The number of aliphatic alcohols analyzed is insufficient to indicate a trend in carcinogenicity. Of the five alcohols analyzed, only 1-[2-(2-methoxy-1-methylethoxy)-1-methylethoxy]-2-propanol was predicted carcinogenic in the female mouse and male rat sub-models, was predicted non-carcinogenic in the male mouse sub-model and was outside the OPS in

the female rat sub-model. The remaining alcohols were either predicted non-carcinogens or were outside the OPS for all four sub-models.

Developmental Toxicity-

All the aliphatic alcohols are predicted negative.

Aromatic Alcohols:

Carcinogenicity-

As in the case of aliphatic alcohols not enough aromatic alcohols were analyzed to realize a trend in carcinogenicity. Most alcohols are predicted as non-carcinogens in all four sub-models with a few exceptions. These exceptions are, 2,4,6-trichlorophenol which is predicted as a carcinogen in the male mouse, 4-(1-methylethyl)-benzene methanol which is predicted positive in the female mouse and female rat sub-models and 2,6-di-tert-butyl-4-nitrophenol which is predicted carcinogenic in the male rat sub-model.

Developmental Toxicity-

With the exception of 4-(1-methylethyl)-benzene methanol all aromatic alcohols are predicted negative.

Ethers:

Aliphatic Ethers:

Carcinogenicity-

The carcinogenic activity is random and spread out for aliphatic ethers, due to the limited number of compounds analyzed. Bromochloromethyl Acetate is predicted as a carcinogen in the female mouse/rat and male mouse. 1-Chloroethanol acetate is predicted as a carcinogen in the male rat sub-model. 1,2-Dichloroethanol acetate is predicted as a carcinogen in the female and

male mice. 2-Methyl-3,3-dichloro-2-propenyl dichloromethylether is predicted positive in the female rat. 1,4-Dioxane is predicted as carcinogenic in female and male mice and male rats, whereas 3-bromopropylchloromethylether is predicted as carcinogenic in all four sub-models. Few compounds are judged to be outside the OPS in various sub-models.

Developmental Toxicity-

All aliphatic ethers are predicted negative for developmental toxicity with the exception of 1-chloroethanol acetate, 3-bromopropylchloromethylether and 1,4-dioxane which are predicted positive.

Aromatic Ethers:

Carcinogenecity-

The only chemical analyzed for carcinogenecity, 1,4-benzodioxin is predicted a non-carcinogen in the male mouse , female and male rat submodels. It was determined to be outside the OPS in the female mouse.

Developmental Toxicity-

1,4-Benzodioxin is predicted negative for developmental toxicity.

Nitriles:

Aliphatic Nitriles:

Carcinogenicity-

The carcinogenic potential of most aliphatics nitriles is predicted as negative by QSAR technique. Carcinogenic activity of aliphatic nitriles seems to be limited to chlorine and bromine substitutions of single and double carbon chains. Cyanogen bromide is predicted as a carcinogen in the female rat. Bromoacetonitrile is predicted positive in female mouse and female rat.

Dichloromethyl cyanide is positive in female mouse. Trichloro- and tribromoacetonitriles are predicted to be carcinogenic in male rats. Bromochloroacetonirile is predicted as a carcinogen in female and male mice, trichloropropenenitrile is predicted positive in the female rat. 2,3-Dichloropropanenitrile and 3,4-dichlorobutanenitrile are predicted as carcinogens in female mice and 3-methylbutane nitrile is predicted positive in the male rat. Few aliphatics nitriles are determined to be outside the OPS.

Developmental Toxicity-

As in the case of halogenated and short chain carboxylic acids, aliphatic nitriles show a tendency toward developmental toxicity. With the exception of bromochloroacetonitrile and 3,4-dichlorobutanenitrile, all other chlorinated and brominated nitriles are predicted as developmental toxicants. Three chlorine substituted nitriles, 2,3-dichloropropanenitrile, cis- and trans-2,3,4-trichloro-2-butenenitrile are determined to be outside the OPS. Of the non-halogenated nitriles, 3-methylbutane nitrile is predicted positive and hepatanenitrile is predicted negative for developmental toxicity.

Aromatic Nitriles:

Carcinogenecity-

The two aromatic nitriles analyzed, are predicted to be non-carcinogenic in all four sub-models.

Developmental Toxicity-

Both aromatic nitriles are predicted negative for developmental toxicity.

Amines:

Aliphatic Amines:

Carcinogenicity-

The number of amines analyzed were too few to recognize a trend in carcinogenicity. For the two amines analyzed, 1-chloro-3,3,3-trichloro-1-propen-1-amine is predicted carcinogenic in the female and male mouse submodels, whereas 5-methyl-3-isoxazolamine is predicted carcinogenic in the male mouse. Both compounds are predicted as non-carcinogens in the remaining sub-models.

Developmental Toxicity-

As in the case of carcinogenicity, no trend can be observed due the fact that only two compounds were analyzed. 1-Chloro-3,3,3-trichloro-1-propen-1-amine is predicted positive, whereas 5-methyl-3-isoxazolamine is predicted negative for developmental toxicity.

Aromatic Amines:

No aromatic Amines were analyzed, either for carcinogenicity or developmental toxicity.

Amides:

Aliphatic Amides:

Carcinogenicity-

Of the two amides analyzed, 2,2-dichloroacetamide is predicted non-carcinogenic in three of the four submodels and is determined to be outside the OPS in the female rat submodel. 2,2,2-Trichloroacetamide is predicted non-carcinogenic in the female mouse and rat submodels and is predicted carcinogenic in the male mouse and rat submodels.

Developmental Toxicity-

2,2-Dichloroacetamide is predicted indeterminant and 2,2,2-trichloroacetamide is predicted as a developmental toxicity.

Aromatic Amides:

No aromatic Amines were analyzed, either for carcinogenicity or developmental toxicity.

Halo & Nitro Compounds:

Aliphatic Alkanes and Alkenes:

Carcinogenicity-

The aliphatic alkanes and alkenes have been subdivided into four groups: (1) The group encompassing halogenated (mainly chlorine and bromine) and nitro methanes, show a carcinogenic trend in the male rat submodel, and a non-carcinogenic trend in the other three submodels for the most part. Methyl chloride and bromodichloronitromethane are predicted as indeterminant and bromochloroiodomethane and nitrodibromomethane are predicted as non-carcinogens in the male rats. Methylene chloride is also predicted as a carcinogen in the male mouse. Dibromomethane and bromodichloromethane are predicted positive in all four submodels. Apart from being carcinogenic in the male rat submodel, bromoform is predicted carcinogenic in female rats, dichloroiodomethane is predicted positive in female mice, bromochloroiodomethane and dbromochloromethane are predicted carcinogenic in female mice and rats and nitrodibromomethane is predicted carcinogenic in the male mouse model. Four of the nitro/halo methanes are determined to be outside the OPS in the female rat submodel (2) The halogenated and nitro alkanes and alkenes (C2 and greater) are non-carcinogenic with a few exceptions. These

exceptions are, 1-chloro-2-ethoxy-2-methoxy ethane, which is predicted as a carcinogen in female and male rats, hexachloroethane is predicted positive in female and male mice and male rats, 1,1,1-tribromo-2-bromo-2-chloroethane is predicted carcinogenic in the female mouse, 3,3,3-trichloro-2-methyl-1-propene is predicted positive in male mice and rats, 2-bromobutane is predicted as a carcinogen in male mice and female rats, 1,1,5,5-tetrachloropentane is predicted carcinogenic in the male rat submodel, 1-hydroxy-3-methyl-2-hexene is predicted positive in female mice and 1-chlorooctane is predicted positive in male rats.

(3) Two compounds identified as outliers, undecane and methane sulfonyl chloride are predicted non-carcinogens in all four submodels with the exception of undecane, which is determined to be outside the OPS in male rats. (4) The cyclic halogenated and non-halogenated alkanes and alkenes show no pattern in carcinogenicity. Tetrachlorocyclopropene and hexachlorocyclopentadiene are predicted as a carcinogens in female rats and cyclododecane is predicted positive in the male mouse and rat submodels.

Developmental Toxicity-

Unlike the carcinogenicity model, there is no specific order to toxicity among the various sub-groups. Methylene Chloride, dibromomethane, trichloronitromethane, bromopicrin, bromodichloronitromethane, 1-ethoxy-1-hydroxymethane, 1-nitro-1,1-dichloroethane, hexachloroethane, 1,2-dichloro-2-methyl butane and methane sulfonyl chloride are predicted as developmental toxicants, whereas the rest are either predicted negative, indeterminant or are determined to be outside the OPS.

Aromatic Compounds:

Carcinogenicity-

Most of the aromatic compounds are predicted to be non-carcinogens with a few exceptions. The few exceptions being, benzene and 1,4-dichlorobenzene which are predicted to be carcinogenic in female and male mice and male rats. 1,3-Dichlorobenzene and 1,2-bis(1-methylethenyl)benzene are predicted positive in the female mouse submodel and, toluene is predicted to be carcinogenic in female rats. Some of these compounds are predicted to be indeterminant or determined to be outside the OPS.

Developmental Toxicity-

All aromatic compounds are predicted negative for developmental toxicity with the exception of benzene which is determined to be outside the OPS and, 1,4-dichlorobenzene which is predicted as indeterminant.

Conclusions:

In addition to our initial agreement with OW of prioritizing the list of DBPs in terms of potential carcinogenicity and developmental toxicity based on probability, NCEA-Cin has categorized these DBPs by chemical class based on functional groups. Based on these classifications, patterns of prediction have been identified that should aid OW in eliminating certain chemical classes based on functional groups. However, in contrasting these predicted data with the available literature, one must follow the criteria used to develop the respective models for all literature published before and after model development. It should be well understood that all QSAR models are closed systems and ultimately should not be used to replace bioassays. However, QSAR predictions are independent of equilibrium changes that are pH dependent, such

as the activity of MX compounds at different pH's in the Ames mutagenicity assay. Using QSAR the authors have been able to distinguish the mutagenic potential of the closed (lactone) and open forms of MX compounds which is not possible under Ames assay conditions.

Developmental toxicity was identified as a health-related endpoint common to the majority of aliphatic mono- and dicarboxylic acids; most aliphatic halogenated and non-halogenated ketones and most aliphatic haloacetonitriles. In the case of the NTP carcinogenicity submodels, most aliphatic aldehydes were identified as likely carcinogens only in the female mouse submodel. The majority of the aliphatic and aromatic dicarboxylic acids were identified as likely carcinogens in the female rat submodel. All other functional groups were for the most part predicted as non-carcinogens in all NTP cancer submodels (male/female rats and mice). An analyses of these QSTR/DBPS results should aid in the prioritization of chemicals to evaluate for these health-related endpoints in the absence of *in vivo* bioassays.

Additional research will include an investigation of which features (descriptors) in a molecule for various chemical classes are responsible for the positive and/or negative predictions and why? In addition, all "similarity search" data for each model and chemical class will be reviewed with the idea of identifying those compounds in the data base that are most similar electronically (in terms of descriptor contribution) to the query compounds and why. Hopefully, this type of information will extend our knowledge and understanding of the structural basis for activity within these classes, whereas a simple list of TOPKAT predictions provides no such insight. Lastly, the authors welcome the opportunity to compare their results to those obtained by other QSAR models.

A.5.3. Summary of DBP-Specific Toxicity Data.

A.5.3.1. Selection of Compounds for Evaluation — The selection of treatment trains for consideration was one factor in the determination of which DBPs were investigated. Other sections of this document have discussed how economic and effectiveness decisions may impact the selection of treatment trains for evaluation and how some specific characteristics of raw water may also impact the formation of DBPs from a given treatment train. We have focused on DBPs identified in a split-sample treatment study of Ohio River water (Miltner et al., 1990). The structures for these chemicals are presented in Figure A.1-2.

A.5.3.2. Individual Chemical Summaries — The goal of this section is to present chemical summaries for several demonstrative chemicals. Due to logistical constraints of this document, and because such summaries for other chemicals may be presently available, the chemicals summarized here are only examples of the toxicologic data that are available on the DBPs. Full chemical summaries should be compiled for the DBPs pertinent to decisions using the CRFM. We have focussed our evaluation on compounds previously identified under a pilot-scale investigation conducted and previously reported (Miltner et al., 1990; see Table A.5-1. Although bromate levels were not measured in Miltner et al. (1990), levels have been estimated for use in this effort (see Chapter 5).

The following summaries provide pertinent details of the chemicals for focus in this comparison. While more exhaustive reviews are available for some compounds, and more data are available for some than others, these sections highlight the important points for consideration. Because of the primary importance of the oral route of exposure for DBPs in drinking water, we have not addressed regulatory concern for exposures other than oral, unless specifically

TABLE A..5-1

EPA - Verified Data Available for Compounds Evaluated in this Case Study

Compound [CASRN]	RfD	Carcinogenic Risk	
		Oral Slope	Unit Risk
Chloroform [67-66-3]	1 E–2 mg/kg/day (09/01/92)	6.1 E–3 (03/01/91)	1.7 E-7
Bromodichloromethane [75-27-4]	2 E–2 mg/kg/day (03/01/91)	6.2 E-2 (03/01/91)	1.8 E-6
Dibromochloromethane [124-48-1]	2 E–2 mg/kg/day (03/01/91)	8.4 E-2 (01/01/92)	2.4 E-6
Bromoform [75-25-2]	2 E–2 mg/kg/day (03/01/91)	7.9 E-3 (01/01/91)	2.3 E- 7
Trichloroacetic Acid [76-03-9]	1 E-1 mg/kg/day (05/06/93)	N/A	N/A
Dichloroacetic Acid [79-43-6]	4 E-3 mg/kg/day (06/15/93)	N/A	N/A
Chloroacetic Acid [79-08-3]	2 E–3 mg/kg/day (02/01/96)	N/A	N/A
Chloral Hydrate [75-87-6]	2 E-3 mg/kg/day (02/01/96)	N/A (?)	N/A (?)
Potassium Bromate [7758-01-2]	N/A	4.9 E-1 (12/11/92)	1.4 E-5

applicable or dictated by lack of orally-relevant data. In reporting reproductive and developmental effects, we have included some data available with whole-embryo culture studies, an *in vitro* design whose results are not deemed applicable in determining regulatory levels (e.g. RfD) of exposure. They are, nonetheless, useful in estimating the potential for such an effect to occur *in vivo*.

TRIHALOMETHANES: The early identification of carbon tetrachloride as a potent liver carcinogen may have heightened the concerns over finding chloroform in drinking water supplies in 1974. This finding stimulated research on THMs which has focused on dose-response relationships, exposure estimates for humans, biomarkers of exposure, and studies of the mechanism(s) underlying cancer. These efforts have spread to other halomethane compounds, resulting in reduced uncertainty about toxicity data generated in rodent species. However, the differences in response noted with different dose regimens/schedules and vehicles complicate the extrapolation of some responses following the administration of bolus doses of THMs. The EPA's Science Advisory Board reviewed the Office of Water's draft Drinking Water Criteria Document (25-26 October, 1990) and advised that the concern over corn oil as vehicle for gavage studies dictated that the hepatocarcinogenic effects (as noted with chloroform) should be utilized only in making a weight-of-evidence classification, and should "be disregarded in making a quantitative estimation of the carcinogenic risk of a trihalomethane." The IRIS file for all three brominated THMs (revised 03/01/91) states that, "No adequate data on the teratogenic or reproductive effects of trihalomethanes are available.", but the IRIS file for chloroform (revised 09/01/92) does not contain such a statement.

Chloroform (CHCl3): Chloroform is the THM which has received the most attention. Care should be taken that chloroform data are not extrapolated without specific and detailed justification. A weight-of-evidence classification of B2 (probable human carcinogen) has been assigned to chloroform. There are no epidemiologic studies for chloroform itself, although chloroform was the major identified DBP in epidemiologic studies which have associated increased incidences of rectal, bladder and colon cancer with drinking water chlorination. Chloroform induces CNS depression and cardiac sensitization, though at doses not likely encountered in drinking water (Bull and Kopfler, 1991). Chloroform is considered highly fetotoxic, but not teratogenic (Schwetz et al., 1974 and Thompson et al., 1974 in U.S. EPA, 1998a); and inhalation exposure produced a dose-dependent increase in post-implantation death and reduced crown-rump length and weight gain in rat pups (ATSDR, 1996). Savitz et al. (1995) used data from case-controlled studies on miscarriages, preterm delivery and low birth weight as related to THM concentration in drinking water to demonstrate that although no dose-response was observed, a significant elevation of miscarriage was present in the highest sextile of THM concentration. Increased association (odds ratios >1.50) of adverse birth outcomes which included low birth weight, "CNS defects", cleft palate and cardiac defects (Bove et al., 1995).

CHCl3: The oral RfD (revised 09/01/92) is based on the finding of fatty cysts in liver of dogs chronically exposed to chloroform in toothpaste (Heywood et al., 1979), and demonstrated a LOAEL of 15 mg/kg/day, which was converted to 12.9 mg/kg/day when dosing schedule was converted to 7 days/week (U.S. EPA, 1998a). Application of uncertainty and modifying factors totaling 1,000 reduce the oral RfD to 10 µg/kg/day (700 µg/day for a 70 Kg human). For carcinogenic risk (updated 03/01/91), an oral slope factor of 6.1x10E-3 per mg/kg/day and a

drinking water unit risk of 1.7x10E-7 per ug/L have been assigned to chloroform. A carcinogenic risk of 1.0x10E-6 results from exposure to a concentration of 6 ug chloroform/L drinking water.

Bromodichloromethane (BDCM): A weight-of-evidence classification of B2 (probable human carcinogen) has been assigned to BDCM. BDCM produces tumors at multiple sites in multiple species, and the kidney tumors produced are independent of alpha-2micro-globulin. There are no epidemiologic studies for BDCM alone, although ecologic and epidemiologic studies which have associated increased incidences of rectal, bladder and colon cancer with drinking water chlorination. Because of the complex mixture of DBPs in drinking water and the co-exposure to other factors which modify cancer formation, these data are insufficient for assessing the carcinogenic risk of BDCM to humans. BDCM is reported to be mutagenic in several in vitro evaluations and is structurally similar to other known animal carcinogens. Klinefelter et al. (1995) reported that the exposure of rats to BDCM in drinking water (at 39 mg/kg/day) sperm velocity was significantly decreased.

BDCM: The oral RfD (revised 03/01/91) is based on the finding of renal cytomegaly in micechronically exposed to BDCM via gavage in corn oil (NTP, 1986), which demonstrated a LOAEL of 17.9 mg/kg/day (U.S. EPA, 1998a). Application of uncertainty and modifying factors totaling 1,000 reduced the oral RfD to 20 µg/kg/day (1400 µg/day for a 70 Kg human). For carcinogenic risk (revised 03/01/93), an oral slope factor of 6.2x10E-2 per mg/kg/day and a drinking water unit risk of 1.8x10E-6 per µg/L have been assigned to BDCM, based on the "linearized multistage model, extra risk". A carcinogenic risk of 1.0x10E-6 results from exposure to a concentration of 0.6 µg BDCM/L drinking water.

Dibromochloromethane (DBCM): A weight-of-evidence classification of C (possible human carcinogen) has been assigned to DBCM. There are no epidemiologic studies for DBCM alone, although ecologic and epidemiologic studies have suggested increased incidences of rectal, bladder and colon cancer associated with drinking water chlorination. DBCM is mutagenic and carcinogenic in male and female mice, however, liver tumors are found only at levels of DBCM which produced liver damage and only with corn oil as vehicle. The IRIS file for all three brominated THMs (revised 03/01/91) states that, "No adequate data on the teratogenic or reproductive effects of trihalomethanes are available", but the IRIS file for chloroform (revised 09/01/92) does not contain such a statement. However, a study by Ruddick et al. (1983) evaluated the developmental toxicity of some THMs. Unfortunately, the low number of fetuses examined from DBCM-exposed dams did not allow the developmental toxicity of DBCM to be confirmed or refuted.

DBCM: The oral RfD (revised 03/01/91) is based on the finding of hepatic lesions in rats exposed subchronically via corn oil gavage (NTP, 1985), which demonstrated a NOEL of 30 mg/kg/day, which was converted to 21.4 mg/kg/day (U.S. EPA, 1998a). A LOAEL of 60 mg/kg/day was observed, which converted to 42.9 mg/kg/day. Application of uncertainty and modifying factors totaling 1,000 (to the NOEL) reduce the oral RfD to 20 µg/kg/day (1400 µg/day for a 70 Kg human). For carcinogenic risk (revised 01/01/92), an oral slope factor of 8.4x10E-2 per mg/kg/day and a drinking water unit risk of 2.4x10E-6 per µg/L have been assigned to DBCM. A carcinogenic risk of 1.0x10E-6 results from exposure to a concentration of 0.4 µg DBCM/L drinking water. There are no published data on teratogenicity or reproductive

effects of trihalomethanes (IRIS, 1998; updated 03/01/91), however, BDCM produced dose-dependent skeletal malformations in rats (Ruddick et al., 1983).

Bromoform (CHBr3): A weight-of-evidence classification of B2 (probable human carcinogen) has been assigned to chloroform. There are no epidemiologic studies for bromoform itself, although ecologic and epidemiologic studies have suggested increased incidences of rectal, bladder and colon cancer associated with drinking water chlorination. Geographic studies have suggested correlations between the levels of trihalomethanes in drinking water and incidences of bladder, colon, rectal and pancreatic cancer in humans. Interpretation of these studies is complicated due to the study design which did not allow for the consideration of other factors which modify carcinogenic response. Bromoform is genotoxic and induced colorectal tumors in mice following intraperitoneal administration and tumors in rats following oral administration. There are no adequate published data on teratogenicity or reproductive effects of trihalomentanes (U.S. EPA, 1998a; chronic oral RfD for bromoform was updated on 03/03/91), although bromoform (and BDCM) produced a dose-dependent increase in skeletal malformations in rats (Ruddick et al., 1983).

CHBr3: The oral RfD (revised 03/01/91) is based on the finding of hepatic lesions in rats exposed subchronically by gavage (NTP, 1989), which demonstrated a NOEL of 25 mg/kg/day, which was converted to 17.9 mg/kg/day (U.S. EPA, 1998a). Application of uncertainty and modifying factors totaling 1,000 reduce the oral RfD to 20 µg/kg/day (1400 µg/day for a 70 Kg human). For carcinogenic risk (revised 01/01/91), an oral slope factor of 7.9x10E-3 per mg/kg/day and a drinking water unit risk of 2.3x10E-7 per µg/L have been assigned to

bromoform. A carcinogenic risk of 1.0x10E-6 results from exposure to a concentration of 4 µg bromoform/L drinking water.

HALOACETIC ACIDS: The primary exposure to TCA and DCA is through drinking water. While these are the only two HAAs for which an IRIS file exists, and they are the most commonly encountered HAA DBPs, their carcinogenic dose-response curves are dissimilar. TCA produces a linear dose-response for liver tumors, while that for DCA demonstrates a distinct dose level, below which tumors are not observed. TCA, DCA and the other HAA DBPs were assessed for *in vitro* developmental effects. Rogers et al. (1995) removed mouse conceptuses (at the 3 to 6 somite stage) and subjected them to whole embryo culture in the presence of mono-, di- and tri-brominated and chlorinated acetic acids for 24 hours. Neural tube defects were observed for all compounds and benchmark concentrations for a 5% increase in defects for the compounds were: dichloroacetic acid, 2452 µM; acetic acid, 1888 µM; tribromoacetic acid, 1403 µM; trichloroacetic acid, 1336 µM; dibromoacetic acid, 162 µM; chloroacetic acid, 91.5 µM; bromoacetic acid, 2.68 µM. While these *in vitro* effects should not be used in risk estimation, they may indicate the qualitative likelihood of an adverse effect. This cannot be further determined from *in vitro* data sets due to lack of physiological parameters such as pharmacokinetics or the inclusion of limits of maternal toxicity. Summary of HAA Reproductive and Developmental Effects are presented in Table A.5-2.

Trichloroacetic Acid (TCA): A weight-of-evidence classification of C (possible human carcinogen) has been assigned to TCA. This has its basis in a lack of human data and the production of tumors in male and female mice, but there is no evidence of carcinogenicity in rats.

TABLE A.5-2

HAA Reproductive and Developmental Effects:

CMPD	Dose Range (mg/kg/day)	Critical Effect	LOAEL (mg/kg/day)	NOAEL (mg/kg/day)	Reference
TCA (rat)	330 - 1800	Cardiovascular, eye	330	-	Smith et al., 1989
DCA (rat)	140 - 400	Cardiovascular	140	14	Smith et al., 1992
DCA (rat)	31.3 - 125	Preputial gland, epididymes	31.3	-	Toth et al., 1992
DCA (mouse)	30 -947	No clear effects	-	947	Narotsky et al., 1996
MCA (rat)	17 - 140	Cardiovascular	140	70	Smith et al., 1990
MBA (rat)	25 - 100	Cardiovascular / craniofacial	100	50	Randall et al., 1991
DBA (rat)	2 - 250	Sperm effects	50	10	Linder et al., 1995
DBA (mouse)	24 - 806	Fetal weight, tail defects	610	392	Narotsky et al., 1996

Genotoxic evaluations have produced mixed results, and TCA does not appear to induce point mutations. No epidemiologic studies have shown an association between exposure to TCA and the production of site-specific tumors. Smith et al. (1989) report a LOAEL of 330 mg/kg/day for developmental effects (resorption, heart and eye defects, decreased fetal weight gain and reduced maternal weight gain) in rats.

TCA: The oral RfD for TCA was not established during the revision accomplished on 01/01/94 (U.S. EPA, 1998a). No quantitative estimates of carcinogenic risk from oral exposure to TCA were established during the revision of the Carcinogenesis Assessment section of IRIS (08/04/93)

(U.S. EPA, 1998a). The Agency is exploring the development of a biologically-based model to accommodate the existing database and other data under development.

Dichloroacetic Acid (DCA): A weight-of-evidence classification of B2 (probable human carcinogen) has been assigned to DCA. This is based on a lack of human carcinogenicity data and an increased incidence of hepatocellular adenoma and carcinomas in female mice. Nodules expected to progress into hepatocellular adenomas and carcinomas were also increased in both rats and mice. No epidemiologic studies have shown an association between exposure to TCA and the production of site-specific tumors. Smith et al. (1992) reported a NOAEL of 14 mg/kg/day for developmental (cardiac) defects.

DCA: An oral RfD is not available on IRIS at this time. There are no quantitative estimates of carcinogenic risk from oral exposure to DCA available at this time (U.S. EPA, 1998a). The Agency is exploring the development of a biologically-based model to accommodate the existing database and other data under development. A subchronic (14-day) dose of 25 mg/kg dose of BAA produced adverse effects on epididymal sperm morphology or histology.

Chloroacetic Acid (MCA): MCA in drinking water at concentrations of up to 1100 mg/L (time-averaged concentration) did not produce liver tumors, pathology, peroxisome or hepatocyte proliferation, or alterations of serum enzymes. Chronic (104 week) exposure of rats to MCA produced incerased spleen weights in all doses 3.5 to 59.9 mg/kg/day) (DeAngelo et al., 1997).

Bromochloroacetic Acid (BCA): The only available published findings with BCA involve a drinking water exposure (21 days) to male mice. Parrish et al. (1996) demonstrated that BCA and DBA produce oxidative stress in liver tissue, as evidenced by hydroxylated DNA adducts,

while TCA and DCA do not. The authors suggest that oxidative damage may modulate chronic toxicity associated with brominated HAAs.

Bromoacetic Acid (MBA): Regulatory levels and toxicity assessments for bromoacetic acid are not available on IRIS. Randall et al. (1991) gavaged pregnant rats with MBA in distilled water and noted a LOAEL of 100 and a NOAEL of 50 mg/kg/day for developmental effects including cardiovascular and craniofacial defects.

Dibromoacetic Acid (DBA): A single dose of 1250 mg/kg DBA produced significant alterations in sperm motility, abnormal sperm head morphology and flagellar degeneration, decreased sperm counts (85% of control) in caput epididymus and decreased serum testosterone (17% of control). Subsequent studies of DBA with male rats indicated a subchronic NOAEL of 10 mg/kg/day with respect to sperm motility of fertility (Linder et al., 1995). Narotsky et al. (1996) reported a delay in partutition in mice daministered DBA by gavage in water; the effect was produced by all doses of DBA (24 to 806 mg/kg/day) and in a dose-dependent manner.

HALOACETONITRILES (HANs): As a group, the data base for haloacetonitriles is not as rich as that for trihalomethanes of haloaceto acids. Due to this constraint, the data presented in this section are combined for ease of presentation.

HAN Toxicity: The target organ(s) for HANs have not been established. Subchronic (90-day) studies with rats dosed via gavage using corn oil vehicle (Hayes et al., 1986) have been performed with DCAN and DBAN. Critical effects of increased liver weight (DCAN) and decreased body weight gain (DBAN) were identified. A NOAEL for DCAN was established at 8 mg/kg/day and a NOAEL for DBAN was established at 6 mg/kg/day. No such values are available for the other HANs. Application of uncertainty factors of 3,000 to these NOAEL values produce “provisional

RfDs" of 3x10E-3 mg/kg/day for DCAN and 2x10E-3 mg/kg/day for DBAN. These values generally agree with provisional reference doses from reproductive and developmental toxicity studies Table A.5-3.

TABLE A.5-3

HAN Reproductive and Developmental Effects:

CMPD	Dose Range (mg/kg/day)	Critical Effect	LOAEL (mg/kg/day)	NOAEL (mg/kg/day)	UF	Provis. RfD	Reference
DCAN	5 - 45	Embryo-lethality	25	15	300	5 x 10E-2	Smith et al., 1989
TCAN	1 - 55	Litter Resorption	7.5	1	300	3 x 10E-3	Smith et al., 1988
BCAN	5 - 65	Cardio-vascular Malforma-tions	5	-	3000	2 x 10E-3	Christ et al., 1995
DBAN	1 – 9.9	None Identified	-	9.9	300	3 x 10E-2	NTP, 1997

All compounds except DBAN (drinking water) were administered in TCAP via gavage to Long-Evans rats over GD 6-18. Values derived from TCAP-based doses may produce somewhat conservative estimates of risk because TCAP stimulates a higher delivery of HANs to the fetus than does corn oil (Gordon et al., 1991).

HAN Mechanistic and Metabolic Considerations: For developmental effects, halogenation seems to be critical, as acetonitrile itself does not produce developmental effects, even at doses which produce maternal toxicity. There is some evidence that interaction with the glutathione-based detoxication system in rodents may modify or modulate some of the toxic effects noted with the HANs. HANs deplete hepatic and GI tract (but not kidney) GSH levels and inhibit GST

activity following their administration (Ahmed et al., 1991); depletion of hepatic GSH prior to HAN (chloroacetonitrile) administration is associated with increased HAN delivery to the fetus (Abdel-Aziz et al., 1993). Although the target organ(s) for HAN toxicity have not been named as such, these effects and the finding of increased liver weight in rats administered DCAN may indicate the liver as a potential target for HAN toxicity. Although there are no published reports indicating the contribution of metabolism to toxicity per se, HANs are metabolized to cyanide and eliminated in the urine as thiocyanates (Lin et al., 1986). Halide displacement or oxidation of a hydrogen atom via mixed function oxidase to produce a hydroxyacetonitrile has been proposed to account for cyanide liberation.

MISCELLANEOUS COMPOUNDS

1,1,1-Trichloropropanone (1,1,1-TCP): 1,1,1-Trichloropropanone (trichloronitromethane; 1,1,1-TCP) is one of the less well-studied DBPs. 1,1,1-TCP is mutagenic *in vitro*, and the mutagenicity of related compounds decreases with increasing degree of chlorination, and is lower for chlorinated than for brominated analogs.

Chloropicrin (CP): Chloropicrin is the most acutely toxic of the DBPs examined in this document. Few studies on its long-term toxicity (e.g. carcinogenicity, NCI, 1978) have been successfully completed, owing to lethality. *In vitro* tests have demonstrated CP's mutagenic potential (gene reversion, primary DNA damage and the induction of sister chromatid exchanges in human lymphocytes. Paucity of reported details preculde the quantitative use of developmental toxicity findings (decreased fetal weight) in rats and rabbits. The acute (4-hour) toxicity of CP is evidenced by temporally biphasic lethality, with animals expiring either within 24 hours, or at approximately 10 days. The LC50 for CP (12 ppm) approximates that of phosgene. A 13-week

exposure of rats to CP by inhalation identified the lung as the primary organ for toxicity (dose-dependent increases in weight and bronchiolar lesions), and identified 0.67 ppm CP as a NOAEL . The relevance of lung as target organ for inhalation exposures is supported by a report of lacrymation, respiratory distress, coughing and bronchitis in humans inhabiting a house which had been previously fumigated with CP (measured concentration of CP was 48 ppb). Exposure of rats to CP via gavage in a 90-day study produced lethality, which was attributed to pulmonary complications from CP aspiration, and necrosis of the stomach as the primary histopathological finding with a NOAEL of 8 mg/kg/day. Although lung was evaluated, no adverse effects were noted at doses of up to 32 mg/kg/day. Together, these data may indicate that CP's toxic is directed at the portal of entry.

Chloral Hydrate (CH): This agent has not been evaluated by the U.S. EPA for evidence of human carcinogenic potential. Sallenfait et al. (1995) exposed chloral hydrate (0, 0.5, 1.0, 1.5, 2.0 and 2.5 mM) to rat embryos in *in vitro* whole embryo culture and noted dose-dependent effects such as decreased crown-rump length, head length and number of somites at doses above 0.5 mM.

CH: The oral RfD (revised 02/01/96) is based on the finding of hepatotoxicity in mice exposed subchronically via drinking water (Sanders et al., 1982), which demonstrated a LOAEL of 15.7 mg/kg/day (U.S. EPA, 1998a). Application of uncertainty and modifying factors totaling 10,000 (to the LOAEL) reduce the oral RfD to 2 µg/kg/day (140 µg/day for a 70 Kg human). Carcinogenic risk for CH has not been established (U.S. EPA, 1998a).

Potassium Bromate (Bromate): To date there have been no surveys reporting concentrations of bromate in drinking water. However, several laboratory and bench-scale pilot treatment studies

have identified factors which contribute to the formation of bromate. Drinking water studies with rats have shown the production of kidney tumors (males and females) and peritoneal mesotheliomas (males only) (Kurokawa et al., 1983). While these results indicate that bromate is a complete carcinogen, additional experiments in this study demonstrated its tumor promoting activity in the kidney, and demonstrated that the lowest dose producing kidney tumors was 6.5 mg/kg/day (doses employed were 0.7, 1.3, 2.5, 5.6, 12.3 and 33.4 mg/kg/day). Interestingly, there was no increase in liver tumors following initiating treatment (with EHEN). Kurata et al. (1992) treated rats with acute doses of bromate followed by promoting doses of barbital sodium to examine tumor initiating activity, but could demonstrate none. The lack of tumor initiating activity may support that longer doses are necessary to initiate tumors or that bromate produces renal tumors through promotional activity. An evaluation of the impact of bromate (either KBrO3 or NaBrO3) indicates that these chemicals, but not KBr induce alpha-2-micro-globulin accumulation in the kidneys of male, but not female, rats (Umemura et al., 1993). These data, coupled with the lack of renal carcinogenicity in mice and hamsters (Kurokawa et al., 1986b; Takamura et al., 1986b), raise the question of the relevancy of bromate-induced renal tumors to the evaluation of cancer risk in humans. The involvement of alpha-2-micro-globulin as an exclusive mechanism of tumorigenicity in rat kidneys is confounded by the finding of renal tumors in female rats (Kurokawa et al., 1983). Alternately, the production of oxidative stress in renal tissue may stimulate cell replication, resulting in tumor promotion (Umemura et al., 1995). Although the finding of renal tumors in female rats may reduce the perceived importance of alpha-2-micro-globulin as an event modifying renal carcinogenicity, its association with male rat kidney tumors may indicate that the mechanism may increase the incidence of tumors in male rats beyond

the incidence in female rats. This may raise questions about the validity of carcinogenic risk estimates for bromate, as they are mainly based on the incidence and dose-response relationship demonstrated for male rat kidney tumors. Consistent with the finding of renal toxicity in rodents, humans acutely exposed to bromate (potassium and/or sodium bromate) in permanent hair wave neutralizing solutions have demonstrated severe renal damage as well as permanent hearing loss. There are no available published reports on the potential of bromate to produce developmental toxicity. Recently, published data (DeAngelo et al., 1998) have confirmed the multi-site carcinogenicity of bromate (in rats). A slight dose-response was noted for kidney tumors in mice. The U.S. EPA (1998a) has considered this evidence supportive of earlier MCL (0.01 mg/L) and MCLG (zero) values.

A.5.4. Developmental and Reproductive Dose-Response Modeling. Table A.5-3 shows chemical names and formulas for the DBPs in the case study along with the availability of developmental and reproductive dose-response data for six of the haloacetic acids (MCA, DCA, TCA, MBA, DBA and BCA), four of the haloacetonitriles (DCAN, TCAN, BCAN and DBAN) and one of the trihalomethanes (BDCM). Seven of these DBPs (MCA, DCA, TCA, MCA, DCAN, TCAN, BCAN) have been subjects of developmental toxicity studies by a single group of investigators, and three (DCA, MBA, DBA) have been the subjects of male reproductive studies by another group of investigators. These studies were all conducted in rats using gavage administration. The results for developmental toxicity were positive. For reproductive toxicity, the dihalogenated haloacetic acids gave positive results, but the monohalogenated acetic acid (MBA) gave negative results. DBAN, was tested in a short-term developmental and reproductive toxicity screening study in rats by the NTP (1992), with negative results. BDCM was tested in a developmental toxicity screening bioassay with positive results. Adequate developmental toxicity data are lacking for DBA and BCA and for DBAN. A surrogate approach seemed appropriate to fill these data gaps, because the available data indicated that developmental toxicity may be common to the haloacetic acid and haloacetonitrile DBPs. As a provisional measure, DCA was selected as a surrogate for the haloacetic acids and TCAN was selected as a surrogate for the haloacetonitriles.

Dose-response modeling was performed on all possible developmental and reproductive endpoints using a linearized multi-stage model with a threshold parameter estimated by the modeling procedure. Modeling results for all model runs can be found in Table A.5-4. For MCA and BDCM, all threshold estimates were above exposure levels for the treatment trains and were

therefore not included in any of the risk estimates. For the other DBPs, the modeling procedure failed to estimate a threshold value for one or more of the data sets such that the threshold was effectively set to zero. For these cases, scientific judgment was used to look across these data sets for the strongest data set and model results, using factors such as evidence of dose-response in the raw data, larger sample sizes, and adequate goodness-of-fit of the model, to choose a dose-response model. For these DBPs, MLE and upper bound slope factors were taken from the modeling results for use in risk estimation.

It may be noted that some of the data in Table A.5-4 are quantal, but other data (body weight, crown-rump length) are continuous, and were converted to quantal (*estimated # of litters affected* in the table) prior to modeling. Conversion of the continuous-response developmental data to quantal form was performed by assuming a normal distribution with a constant variance across dose groups for the response, and 5% background response rate. Because individual animal data were not available, the number of responders in each dose group was estimated by first establishing a critical value representing the point above (or below, depending on the direction of adverse response) which 5% of the control group lies. Then for each dose group the proportion exceeding this critical value was estimated. This proportion was applied to the number of animals in the dose group to determine the number of responders. The doses were converted to equivalent human doses using the scaling factor of body weight 2/3 power, consistent with the conversions used for the Agency verified cancer slope factors used in the case study. [Note: The U.S. EPA 1996 Proposed Cancer Guidelines have proposed using a scaling factor of body weight to the 3/4 power. Future applications of the CRFM should employ this assumption.]

Table A.5-3

Availability of Developmental and Reproductive Dose-Response Data

Chemical			Developmental Toxicity[a]	Reproductive Toxicity[a]
Haloacetic Acids				
$ClCH_2COOH$	Monochloroacetic Acid	MCA	y, (+)	
$Cl_2CHCOOH$	Dichloroacetic Acid	DCA	y, +	y, +
Cl_3CCOOH	Trichloroacetic Acid	TCA	y, +	
$BrCH_2COOH$	Monobromoacetic Acid	MBA	y, +	y, -
$Br_2CHCOOH$	Dibromoacetic Acid	DBA		y, +
BrClCHCOOH	Bromochloroacetic Acid	BCA		
Haloacetonitriles				
Cl_2CHCN	Dichloroacetonitrile	DCAN	y, +	
Cl_3CCN	Trichloroacetonitrile	TCAN	y, +	
BrClCHCN	Bromochloroacetonitrile	BCAN	y, +	
Br_2CHCN	Dibromoacetonitrile	DBAN	y,(-)[b]	y, (-)[b]
Trihalomethanes				
$CHBrCl_2$	Bromodichloromethane	BDCM	y, +	

[a]Data are from gavage studies in rats unless otherwise noted.
[b]Data are from a screening-level drinking water study in rats.
y = yes, adequate data available
+ = results were positive for adverse effect
- = results were negative for adverse effect
(+) = results were marginally positive
(-) = results were negative, but a toxicity-based MTD could not be achieved due to taste aversion and consequent refusal to drink higher concentrations of the chemical, and this was a short-term screening study

Table A.5-4

Modeling Results using $BW^{2/3}$ Scaling Factor*

Data Set [note all are for rats except when noted (DCA, Cicmanec et al.)]	Equiv Human ED_{01} mg/kg-d	Equiv Human ED_{10} mg/kg-d	Threshold mg/kg-d
MCA Smith et al , Fetal body weight	18.9	26.7	11.2
MCA Smith et al , Crown-rump length	15.7	20.2	11.2
MCA Smith et al, Visceral Malformations	16.5	21.8	11.2
DCA Smith et al , Fetal body weight - male	4.7	27.3	2.2
DCA Smith et al , Fetal body weight - female	18.6	40.4	16.3
DCA Smith et al , Crown-rump length - male	5.1	36.2	1.0
DCA Smith et al , Crown-rump length - female	5.1	36.2	1.0
DCA Smith et al , Visceral malformations Total	1.2	12.2	0
DCA Smith et al , Visceral malformations Cardiovascular	1.7	17.6	0
TCA Smith et al , Complete litter resorption	110.5	143.2	106.3
TCA Smith et al , % Postimplantation loss/litter	51.1	88.9	46.8
TCA Smith et al , Fetal body weight - male	0.5	5.2	0
TCA Smith et al , Fetal body weight - female	0.6	6.0	0
TCA Smith et al , Fetal crown-rump length - male	16.2	26.8	15.0
TCA Smith et al , Fetal crown-rump length - female	22.9	37.9	21.4
TCA Smith et al , Visceral malformations Total	25.7	32.2	25.0
TCA Smith et al , Visceral malformations Cardiovascular, total	11.9	23.4	10.7
TCA Smith et al , Visceral malformations Levacardia	1.3	13.8	0

Data Set [note all are for rats except when noted (DCA, Cicmanec et al.)]	Equiv Human ED_{01} mg/kg-d	Equiv Human ED_{10} mg/kg-d	Threshold mg/kg-d
TCA Smith et al , Skeletal malformations	129.7	145.3	128.0
MBA Randall et al., Fetal body weight	4.4	13.7	3.4
MBA Randall et al., Fetal crown-rump length	1.2	12.5	0
MBA Randall et al., Visceral malformations (% affected/litter)	10.2	15.5	6.1
DCA Cicmanec et al , Testicular lesions: degeneration, **dog**	Failed to converge		
DCA Linder et al , Number caput sperm	33.3	74.6	28.8
DCA Linder et al , Number cauda sperm	Failed to converge		
DCA Linder et al., % Motile sperm	12.6	16.5	9.7
DCA Linder et al., Progressive motility	10.8	15.4	9.7
DCA Linder et al., Testicular histopathology: Faulty spermiation	Failed to converge		
DBA Linder et al , Number caput sperm	5.6	7.7	5.4
DBA Linder et al , Number cauda sperm	0.4	4.2	0
DBA Linder et al , % Motile sperm	9.4	13.9	5.4
DBA Linder et al , Progressive motility	9.4	13.9	5.4
DBA Linder et al , Retention Stage IX spermatids per tubule	0.1	1.1	0
DCAN Smith et al , Complete litter resorption	2.4	3.2	2.3
DCAN Smith et al , % Postimplantation loss/litter	2.3	3.6	1.9
DCAN Smith et al , Fetal body weight - male	2.1	4.3	0.8
DCAN Smith et al , Fetal body weight - female	2.6	3.6	2.4
DCAN Smith et al , Fetal Crown-rump length - male	2.8	4.1	2.4

Data Set [note all are for rats except when noted (DCA, Cicmanec et al.)]	Equiv Human ED_{01} mg/kg-d	Equiv Human ED_{10} mg/kg-d	Threshold mg/kg-d
DCAN Smith et al , Fetal Crown-rump length - female	2.3	3.4	2.2
DCAN Smith et al , Visceral malformations Total	1.5	2.3	1.5
DCAN Smith et al , Visceral malformations Cardiovascular	0.2	1.8	0
DCAN Smith et al , Visceral malformations Urogenital	0.9	2.3	0.8
DCAN Smith et al , Skeletal malformations	1.1	3.2	0.8
TCAN Smith et al , Complete litter resorption	0.24	0.97	0.16
TCAN Smith et al , % Postimplantation loss/litter	0.5	1.2	0.4
TCAN Smith et al , Fetal body weight - male	0.2	1.7	0
TCAN Smith et al , Fetal body weight - female	0.1	1.1	0
TCAN Smith et al , Visceral malformations Total	0.05	0.5	0
TCAN Smith et al , Visceral malformations Cardiovascular	0.09	0.9	0
TCAN Smith et al , Visceral malformations Urogenital	0.06	0.7	0
BCAN Christ et al, Complete litter resorption	1.1	3.7	0.8
BCAN Christ et al, % Postimplantation loss/litter	0.6	6.5	0
BCAN Christ et al, Fetal body weight - male	0.8	2.0	0.6
BCAN Christ et al, Fetal body weight - female	1.0	2.8	0.8
BCAN Christ et al, Fetal crown-rump length - male	0.5	4.8	0
BCAN Christ et al, Fetal crown-rump length - female	0.2	1.9	0
BCAN Christ et al, Visceral malformations Total	0.06	0.6	0
BCAN Christ et al, Visceral malformations Cardiovascular	0.07	0.7	0
BCAN Christ et al, Visceral malformations Urogenital	0.5	1.9	0.4

Data Set [note all are for rats except when noted (DCA, Cicmanec et al.)]	Equiv Human ED_{01} mg/kg-d	Equiv Human ED_{10} mg/kg-d	Threshold mg/kg-d
BCAN Christ et al, Skeletal malformations	1.0	3.4	0.8
BDCM Narotsky et al, Complete litter resorption	3.8	6.1	3.5

[+]High Dose Dropped

*Dose conversions performed prior to modeling.
Dose Conversion Factor to convert animal dose in mg/kg-day to equivalent human dose in mg/kg-day = $(BW_a/BW_h)^{1/3}$

For rat developmental data (HAAs, HANs): = 0.16
(BDCM): = 0.14

*For male **rat** reproductive data:* = 0.18

*For male **dog** reproductive data:* = 0.52

BW_a = animal body weight in kg = 0.30 kg for rat developmental data on HAAs and HANs, 0.21 kg for rat developmental data on BDCM, 0.41-0.42 kg for male rat reproductive data, 10 kg for male dog reproductive data
BW_h = human body weight = 70 kg

A.6. HUMAN DATA ON HEALTH RISKS FROM EXPOSURE TO DISINFECTED DRINKING WATER: EPIDEMIOLOGIC STUDIES OF CANCER AND REPRODUCTIVE/DEVELOPMENTAL EFFECTS

A.6.1. Introduction. Both epidemiological and toxicological methods have been used to assess human health risks from exposure to disinfected drinking water. Results from experiments in animals must be extrapolated from exposures that are several orders of magnitude higher than actual human exposures, and synergistic and antagonistic effects of mixtures of chemicals are not taken into account when evaluating the carcinogenic risks of individual DBPs. Epidemiology offers the opportunity to study directly mixtures of chemicals at relevant exposures in humans. The studies must be properly designed, conducted with minimal systematic bias, and should be sufficiently large to provide information to confidently judge the impact of observed associations.

Since the early 1970's, a large number of epidemiologic studies of varying design and quality have been published in the scientific literature. The studies have focused almost exclusively on chlorinated drinking water and its association with cancer rather than on individual chemical exposures. Reproductive and developmental epidemiologic studies on this topic first appeared in the literature in the late 1980's. However, only recently have investigators collected information to quantitatively estimate exposures to individuals from different chemical families and species of DBPs and begun to study disinfectants other than chlorine.

The purpose of this section is to provide a brief overview of the existing epidemiologic literature suggesting a potential hazard from exposure to disinfected drinking water and and its associated DBPs.

A.6.2. Cancer Studies. Several types of epidemiological studies have been conducted to assess the association between cancer and chlorinated drinking water. Ecological (Harris, 1974; Page et

al., 1976; Cantor et al., 1978; Hogan et al., 1979; Carlo and Mettlin, 1980; Tuthill and Moore, 1980; Wigle et al., 1986; Flaten, 1992), cohort (Wilkins and Comstock, 1981; Doyle et al., 1997), and case-control designs (e.g., Brenniman et al., 1980; Cragle et al., 1985; Gottlieb et al., 1981, 1982; Cantor et al., 1987, 1998; Ijsselmuiden et al., 1992; McGeehin et al., 1993; King and Marrett, 1996; Hildesheim et al., 1998; Young et al., 1987) have evaluated both incident and decedent cases. These studies differ in their basic approach and the evidence they can provide about the possible causality of an epidemiological association between chlorinated drinking water and cancer. These studies are not reviewed in detail in this document. However, a summary of the more methodologically sound studies, e.g, those based on incident cases, and having interviews and individual exposure estimates, is provided in Table A.6-1.

Because meta-analytic methods can be used to quantitatively summarize a body of literature and provide a single point estimate of effect for a body of literature, it seems logical to pursue such an estimate for application in this cost effectiveness case study. In an attempt to quantitatively review the literature, Morris et al. (1992) presented an aggregate meta-analysis of the published epidemiologic literature relating to water chlorination and cancer. They identified 10 articles published between 1966 and 1991 that evaluated exposure to chlorinated water and cancer at the level of the individual (ecological studies were excluded). The authors of these 10 articles evaluated a dozen different cancer sites and reported overall odds ratios (OR) ranging from less than one to almost three. The cancer sites most frequently examined were bladder and colon (7 articles each), followed by stomach, rectum, and pancreas (6 articles each). Morris et al.

TABLE A.6-1

SUMMARY OF INTERVIEW-BASED CASE-CONTROL AND COHORT STUDIES*

Overview	Population	Exposure Assessment	Analysis
Reference: Cantor et al., 1998 *Type of study*: case-control (incidence) *Cancer site(s)*: bladder; 5 other sites also studied	*Population base*: residents of Iowa. *Cases*: 1,123 bladder cancers, ages 40-85 yrs., histological confirmation of all cases, identified primarily through State Health Registry of Iowa *Controls*: 1,983 age-gender-race frequency matched sample of the general population; no previous cancer diagnosis	*Exposure measure*: mailed questionnaire obtained estimates of fluid and tap water consumption, residential and water source history; duration of use of chlorinated surface water, unchlorinated ground water, fluid and tap water consumption. *Ascertainment of D/DBPs*: water source and treatment from water company records and recent measures of water contaminants such as THMs.	*Method*: logistic regression adjusted for potential confounders, such as age, farm occupation, diet, physical activity, cigarette smoking. *Findings*: little overall association between bladder cancer risk and exposure to chlorination by-products. Bladder cancer risk increased with exposure duration , but opposite trends were found in males and females; further analyses that included total lifetime and average lifetime TTHM levels show all risk increases are apparently restricted to male smokers.

Overview	Population	Exposure Assessment	Analysis
Reference: Cantor et al., 1987 *Type of study*: case-control (incidence) *Cancer site(s)*: Bladder (National Bladder Cancer Study)	*Population base*: white U.S. residents in 10 locations. *Cases*: 2,805, age 21-84, diagnosed 1977-1978, identified from tumor registries. *Controls*: 5,258 from general population; frequency matched to cases by sex, age, and geographic area; identified through phone sampling (to age 64) or sample of Medicareroster (age 65 and over).	*Exposure measure*: duration of use of chlorinated surface water vs. nonchlorinated ground water; tap water consumption. *Ascertainment of D/DBPs*: information on water source (surface vs. ground) and chlorination from survey of utilities; residential history, and level of consumption of tap water and beverages, by personal interview.	*Method*: logistic regression; adjusted for age, gender, study area, smoking, usual or high-risk occupation, and urbanicity of place of longest residence. *Findings*: for whites with >59 years exposure to chlorinated water overall OR = 1.1 (0.8-1.5), non-smokers OR = 2.3 (1.3-4.2), current smokers OR = 0.6 (0.3-1.2); for whites with 40-59 years exposure to chlorinated water overall OR = 1.0 (0.8-1.3), non-smokers OR = 1.4 (0.9-2.3), current smokers OR = 0.7 (0.5-1.2); for those with 40-59 years of chlorinated surface water use, OR for highest quintile of tap water consumption relative to lowest quintile = 1.7 (p for trend = 0.006); for those with $\geq$60 years of use, OR = 2.0 (p for trend = 0.014).

Overview	Population	Exposure Assessment	Analysis
Reference: McGeehin et al., 1993 *Type of study*: case-control *Cancer site(s)*: bladder (incidence)	*Population base*: white Colorado residents from the State Cancer Registry. *Cases*: 327. *Controls*: 261 frequency matched by gender and 5-year age group randomly selected from cancer registry during same period, excluding lung and colorectal cancers.	*Exposure measure*: residential history and level of tap water consumption; duration of use of chlorinated/chloraminated surface water, chlorinated/unchlorinated ground water, bottled water; tap water consumption. *Ascertainment of D/DBPs*: information on water source and chlorination or chloramination from site visit to water utilities; water quality data collected for total THMs, chlorine residual, and nitrates.	*Method*: logistic regression adjusted for smoking, coffee, history of kidney stones and familial bladder cancer, and occupation. *Findings*: OR for bladder cancer = 1.8 (1.1-2.9) for >30 years' exposure to chlorinated water. Cases consumed more tap water per day than controls ($p<0.01$); OR for bladder cancer = 2.0 (1.1-2.8) for cases consuming >5 glasses of tap water. Risk of bladder cancer decreased with increased duration of exposure to chloraminated surface water ($p<0.01$); OR = 0.6 (0.4-1.0) for those consuming chloraminated water >40 years. Level of total THMs, residual chlorine, or nitrates not associated with bladder cancer risk controlling for years of exposure.

Overview	Population	Exposure Assessment	Analysis
Reference: Freedman et al., 1997 *Type of study*: nested case-control *Cancer site(s)*: bladder (incidence)	*Population base*: white residents of Washington County, MD, included in 1975 county census. *Cases*: 294 new cases reported to Washington County cancer registry, 1975-1992. *Controls*: 2,326 frequency matched by age and gender, randomly selected from 1975 census.	*Exposure measure*: chlorinated vs. nonchlorinated drinking water (Municipal, vs. nonmunicipal source); fluid consumption not obtained. *Ascertainment of D/DBPs*: information on water treatment from prior study; drinking water source obtained in 1975 county census.	*Method*: logistic regression adjusted for age, sex, smoking level and history, urbanicity, marital status, education. *Findings*: OR = 1.2 (0.9-1.6) using 1975 measure of exposure to chlorinated vs. nonchlorinated water; slight gradient of incresing risk with increasing duration of exposure noted only among smokers; further stratification by gender showed elevated ORs to be restricted to subcategory of male smokers.

Overview	Population	Exposure Assessment	Analysis
Reference: King and Marrett, 1996 *Type of study*: case-control *Cancer site(s)*: bladder (incidence); colon and rectal cancers also studied, but results not yet reported	*Population base*: residents of Ontario, Canada, ages 25-74 years. *Cases*: 696. *Controls*: 1545 age-gender frequency matched sample of the general population from households randomly selected from residential phone listings; controls also used to study colon and rectal cancer and age-gender distribution based on that expected for all 3 sites combined.	*Exposure measure*: mailed questionnaire/telephone interview obtained estimates of fluid and tap water consumption, residential and water source history: duration of use of chlorinated surface water, unchlorinated ground water, fluid and tap water consumption. *Ascertainment of D/DBPs*: water source and treatment from water company records and questionnaire; combined with model to estimate past total THMs summer levels (annual peak value) by year.	*Method*: logistic regression adjusted for age, gender, education, cigarette smoking, caloric intake. *Findings*: bladder cancer risk increased with increasing number of years exposure to chlorinated surface water, but was statistically significant only for lengthy exposures. OR for bladder cancer = 1.41 (1.09-1.81) for >34 years exposure to chlorinated surface water compared to <10 years exposure. OR for bladder cancer =1.44 (1.10-1.88) for exposure to >1956 ug/l-years THMs compared to <584 ug/l-years; risk increases by 11% with each 1,000 ug/L THMS-years. Results provide no support for an interaction between volume of water consumed and years of exposure to THMs level >49 ug/L. Among those with relatively homogenous exposures for >29 years, trend for increased bladder cancer risk with increased THMs levels (p=0.006) and OR for bladder cancer = 1.39 (1.09-1.79) for chlorinated surface water compared to ground water.

Overview	Population	Exposure Assessment	Analysis
Reference: Young et al., 1987 *Type of study*: case-control *Cancer site(s)*: colon (incidence)	*Population base*: WI residents, age 35-90. *Cases*: 347 new cases reported to WI Cancer Registry over 2-year period. *Controls*: 639 new cases of non-gastrointestinal/urinary tract cancer reported to registry; also 611 population controls, a random sample of WI drivers.	*Exposure measure*: high or medium vs. low lifetime exposure (and period-specific exposure) to total THMs. *Ascertainment of D/DBPs*: water source and treatment from water company records and questionnaire; combined with model to estimate past total THM levels by year; residential history, drinking water sources, and use of tap water from self-administered questionnaire.	*Method*: logistic regression; adjusted for age, sex, and urbanicity of residence. *Findings*: for lifetime exposure: for high exposure group, OR = 0.93 (0.55-1.57) using cancer controls and 0.73 (0.44-1.21) using population controls; for medium-exposure group, OR = 1.05 (0.66-1.68) using cancer controls and 1.10 (0.68-1.78) using population controls.
Reference: Cragle et al., 1985 *Type of study*: case-control *Cancer site(s)*: colon (incidence)	*Population base*: white NC residents with residency $\geq$10 years. *Cases*: 200 new cases over 18-month period from 7 NC hospitals, resident in NC $\geq$10 years. *Controls*: 407 non-cancer hospital patients with admission date nearest diagnosis date of case, matched to case in age, race, gender, vital status, and hospital.	*Exposure measure*: duration of exposure to chlorinated drinking water (none vs. 1-15 years vs. 16-25 years), 1953-1978. *Ascertainment of D/DBPs*: queried local water treatment plants about water source and treatment; residential history by questionnaire (phone or self-administered).	*Method*: logistic regression adjusted for sex, age, genetic risk, dietary fiber, region of NC, urban residence, smoking, alcohol use, education, and number of pregnancies. *Findings*: for age 60: OR = 1.38 (1.10-1.72) for longer exposure and 1.18 (0.94-1.47) for shorter exposure; for age 70: OR = 2.15 (1.70-2.69) and 1.47 (1.16-1.84); for age 80: OR = 3.36 (2.41-4.61) and 1.83 (1.32-2.53).

Overview	Population	Exposure Assessment	Analysis
Reference: Hildesheim, 1998 *Type of study*: case-control *Cancer site(s)*: colon and rectal cancers (incidence)	*Population base*: residents of Iowa *Cases*:560 colon cancers, 537 rectal cancers ages 40-85 yrs., histological confirmation of all cases, identified primarily through State Health Registry of Iowa *Controls*: 1983 age-gender-race frequency matched sample of the general population ; no previous cancer diagnosis Cases and controls studies had at leasts70% of lifetime drinking water exposures documented	*Exposure measure*: mailed questionnaire obtained estimates of fluid and tap water consumption, residential and water source history; duration of use of chlorinated surface water, unchlorinated ground water, fluid and tap water consumption. *Ascertainment of D/DBPs*: water source and treatment from water company records and recent measures of water contaminants such as THMs.	*Method*: logistic regression adjusted for potential confounders, such as age, farm occupation, diet, physical activity, cigarette smoking, urbanicity. *Findings*: No association between colon cancer and estimates of past chlorination by-product exposure. Rectal cancer risk increased siginificantly with duration of exposure to chlorinated surface water and increasing lifetime THMs exposure; larger odds ratios found among those with low fiber intake and low levels of physical activity.

Overview	Population	Exposure Assessment	Analysis
Reference: Doyle, 1997 *Type of study*: cohort *Cancer site(s)*: Eleven anatomic sites including bladder, colon, rectum, liver, kidney, pancreas, breast (incidence)	*Population base*: 36,127 female residents of Iowa in Women's Study, ages 55-69; followed for cancer incidence and mortality thru 12/93 *Exposed*: Women served by 100% surface water or mixed surface and groundwater *Unexposed*: Women served by 100% groundwater (referent category)	*Exposure measure*: mailed questionnaire for drinking water source; other info obtained at baseline 1986 via questionairre *Ascertainment of D/DBPs*: mailed questionnaire for drinking water source; water company records and statewide survey used for recent measures of water contaminants for 4 specific THMs	*Method*: Cox proportional hazards regression, adjusting for age, smoking, education, physical activity, vegetable and fruit intake, total calorie intake, and anthropomorphic measures. *Findings*: Compared to consumers of 100% ground water, RR for colon cancer were 1.67 (95% CI=1.07, 1.52) for consumers of 100% surface water, 1.52 (95% CI=1.08, 2.14) for consumers of mixed ground and surface sources; elevated risk for combined total cancer also noted; significant dose-response noted for colon with increasing chloroform exposure; no elevated risks observed for rectal cancer; bladder cancer RR inconsistent.

Studies with historical water exposure information; 95 percent confidence interval for OR in parentheses unless otherwise noted.

generated summary (weighted average) OR and 95% confidence interval estimates for each of the 12 cancer sites and reported significantly elevated ORs of 1.21 (95% CI = 1.09-1.34) and 1.38 (95% CI = 1.01-1.87) for bladder and rectal cancer, respectively. The OR for all cancer sites combined was reported to be 1.15 (95% CI = 1.09-1.20).

At the request of U.S. EPA, this work was independently evaluated and reanalyzed by Dr. Charles Poole of Boston University (Poole, 1997). Poole found that there was considerable heterogeneity among these data and that there was evidence of publication bias within this body of literature. He also found that the aggregate estimates reported by Morris et al. (1992) were unstable and were sensitive to reasonable changes in the analytical methods and to the addition or deletion of a single study. For these and other reasons, Poole recommended that these data not be combined into a single summary estimate and that they had limited utility for risk assessment purposes. Furthermore, he concluded that the issue of whether or not water chlorination caused cancer was still an open question (Poole, 1997). Because of these findings, a summary OR from which probabilities of cancer occurrence in relation to DBP exposure could be derived is not currently available, and has not been used in the current case study. Several additional studies of cancer and exposure to D/DBPs have been published since the conclusion of the analysis by Poole. The U.S. EPA is currently developing a further quantitative analysis of the available epidemiologic literature which is designed to include an updated literature search extended to the present time and an evaluation of the body of relevent studies on the basis of more meaningful exposure groupings. Of particular interest will be creating study groupings formed from the perspective of the type of source water, specific treatment technologies, and type and amount of the specific chemical byproducts present in the delivered water. If the epidemiologic studies can

be meaningfully categorized and summarized in this way (including the determination that aggregation of study results is appropriate), the human data can be used in future applications of this cost effectiveness methodology.

Current toxicological assessment, based on only a small percentage of DBPs with adequate toxicological data, suggest a relatively small risk from the chlorination of drinking water. Additional epidemiological evidence is needed to clarify both the causal nature of the observed associations between chlorinated water and any site-specific cancers, and the magnitude of change in risk if it is real. In addition, toxicological studies implicate different target organs (primarily liver and kidney) than epidemiological studies (primarily bladder and colon/rectum), yet the basis of these differing responses has not received serious study.

A.6.3. Reproductive/Developmental Studies. Although fewer in number than the body of cancer literature, epidemiological studies of reproductive and developmental outcomes have been performed. The outcomes considered have included stillbirth, spontaneous abortion (Aschengrau et al., 1989; Savitz et al., 1995; Swan et al., 1998; Waller et al., 1998), low birth weight (<2500 g) (Lynberg, 1987; Kramer et al., 1992; Savitz et al., 1995), intrauterine growth retardation (Kramer et al., 1992), somatic effects (Kanitz et al., 1996), and birth defects including cardiac and neural tube defects (Bove et al., 1995; Aschengrau et al., 1993). Almost all examined multiple outcomes and multiple exposure variables. In 1993, an expert scientific panel convened by EPA and ILSI (ILSI, 1993; Reif et al., 1996) reviewed the epidemiologic literature on reproductive/developmental endpoints and DBP and disinfectant exposures. The panel concluded that the research in this area was in a very early and evolving stage and that the studies should be viewed as preliminary. A second expert panel convened by EPA in 1997 reviewed more recently

published work, e.g., Kanitz et al. (1996) and Savitz et al. (1995), and reached a similar conclusion. The panel stated that "The results of epidemiological studies reported to date do not provide compelling evidence about the association of adverse outcomes of pregnancy and DBPs. Associations found in most studies may be due to one or more sources of bias or residual confounding from unidentified risk factors." (U.S. EPA, 1998c, p. 2-15).

This same panel also reviewed an unpublished version of a study by Waller et al. that has been published subsequently as two companion articles (Swan et al., 1998; Waller et al., 1998). This well-designed and -conducted prospective cohort study from California reported an increased risk of spontaneous abortion associated with high consumption of drinking water with high levels of total THMs and with BDCM exposures >18 µg/L, controlling for other THMs. No information was available for other DBPs of interest, including the HAAs. The expert panel found these results to be provocative, but noted that this is the first and only study to date to have reported these specific findings. The experts encouraged further research and specifically recommended that efforts be made both to replicate the work of Waller et al. (1998) and Swan et al. (1998) in other geographic areas, and to evaluate additional drinking water exposures in the same cohort (U.S. EPA, 1998c, p. 3-2).

Because the human data base is both uncertain and sparse, the epidemiologic studies of reproductive and/or developmental outcomes have not been used in the current case study. Instead, the results from laboratory animal studies of individual DBPs have been combined to produce a "non-specific" estimate of the reproductive/developmental risk produced by each water treatment option (see Chapter 5.3.1.) Similar to the cancer studies described in section A.6.2 above, additional analyses of the human studies on reproductive and/or developmental outcomes

will also be developed and will be applied in future applications of this cost-effectiveness methodology.

A.6.4. Summary/Conclusions. Assessing the potential health risks to humans from exposure to DBPs is plagued by inadequate data and inconsistencies in the available epidemiologic literature. Many of the studies reporting associations between chlorinated water and/or DBPs and various cancers or adverse reproductive/developmental outcomes may have biases that limit the interpretability of their findings. Moreover, the studies vary according to the amount of information available on exposure to chlorinated byproducts, specific DBPs, and other water contaminants.

The above-noted inconsistencies and methodologic problems in the epidemiologic studies make it difficult to select valid and reliable data points from these human studies for input into the case study. Various alternative approaches for quantifying the epidemiologic literature are being pursued currently and, at a minimum, these data may be used as part of a future sensitivity analysis for the case study.

A.7. APPLICATION OF METHODOLOGY TO IN-HOME DRINKING WATER TREATMENT SYSTEM

Placement of in-home drinking water treatment systems in the homes of immunocompromised individuals is an alternative to the construction of a new treatment plants which have a greater anti-microbial efficacy. This section compares the expected utility of adding an in-home reverse osmosis treatment system for drinking water (point-of-use/single tap) to the homes of immunocompromised members of a population, with the expected utility of constructing an ozone/chlorine treatment system for the entire population (i.e., 500,000) served by such a system. This example evaluates the cost of adding these in-home systems per quality-adjusted life year from a societal perspective. The immunocompromised subpopulation within a community is thought to be at greater risk for infection, disease, and death as a result of exposure to protozoa in drinking water than the rest of the population (Hoxie et al., 1997). Additionally, the Centers for Disease Control has issued guidance regarding water filters for use by immunocompromised persons (CDC, 1995). This application only examines the removal of *Cryptosporidium* oocystsand their oocysts by reverse osmosis and does not evaluate the impact of changes in disinfectant and DBP levels in the water.

Protozoa in drinking water may be the result of 1) a lack of effectiveness of the treatment system against these agents, 2)breakthroughs in the treatment train, or 3)within delivery system contamination. The qualitative health benefits of adding a point-of-use reverse osmosis drinking water system for immunocompromised individuals has been explored (Juranek, 1995). The article notes that a high level of protection may be conferred as a result of removal of *Cryptosporidium* oocysts by these systems and warns that manufacturers instructions should be carefully followed.

Several simplifying assumptions are employed in this example application:

- an estimate of adult and children infected with Acquired Immunodeficiency Syndrome (AIDS) adequately accounts for all individuals with compromised immune systems,
- absolute removal of the oocysts by the reverse osmosis in-home systems,
- no change in the DBP levels in the drinking water as a result of in-home system
- as a result of preventing *Cryptosporidium* exposures to the susceptible population, essentially no deaths result from sequale of *Cryptosporidium* infections, and finally,
- estimates of the initial and installation costs of the reverse osmosis systems as well the annual costs adequately represent actual costs incurred by households employing these units.

A.7.1. Estimated Size of the Immunocompromised Subpopulation. The immuno-ompromised subpopulation consists of individuals receiving immunosuppresive therapies, individuals with Acquired Immunodeficiency Syndrome (AIDS), and other individuals with impaired immune functions. For this example the number of individuals with AIDS will be used as an estimate of the entire immunocompromised subpopulation. The CDC (1997) states that there are 26.7 U.S. AIDS cases per 100,000 population. These case rate varies by gender, age, and race. Certain metropolitan areas have higher annual rates than the national average. For example, New York City, NY has the highest rate in the United States at 117.2/100,000. Other metropolitan areas have lower yearly rates; for example, the rates in Denver, CO and Little Rock, AR are 17.5/100,000 (National Center for Health Statistics, 1997; see also Perz et al., 1998). For

this example, it is assumed that each immunocompromised individual resides in a separate household.

A.7.2. Point-of -Use Drinking Water Treatment Technology, Effectiveness, and Cost.

Reverse osmosis is an increasingly popular point-of-use drinking water treatment method, which removes chemicals and biologic agents based on the molecular weights of chemicals. In the reverse osmosis process, feed (i.e., incoming) water passes through a semi-permeable membrane under pressure. The pressure to the incoming water must be greater than the finished water to reverse the osmotic potential of the finished water and, hence, drive the system. When operating properly, the reverse osmosis process removes 90-98% of inorganics ions, 98-100% of dissolved organic contaminants with a molecular weight greater than 200 daltons and 100% of the biologic agents (Lykins et al.,1992: p. 49-54). In 1995 the CDC stated,

> "Only microstraining filters capable of removing particles $\leq$1 µm in size should be used by immunocompromised persons and other persons who choose to use a personal-use filter (i.e., home or office water filters) to reduce the risk for transmission of *Cryptosporidium*. Filters in this category that provide the greatest certainty of *Cryptosporidium* removal include those that produce water by reverse osmosis, those labeled according to filter manufacturing industry standards as "Absolute" 1 µm filters, and those labeled as meeting American National Standards Institute (ANSI)/NSF (formerly the National Sanitation Foundation) International standard #53 for Cyst Removal."

For intact systems the expectation is an absolute removal of protozoa from feed waters resulting in protozoan free finished water; however, microbiologic agents have been observed in the finished drinking water produced during system malfunctions. For example, leak out, also known as pass through, of biologic agents can occur through improper installation of the system or a loss of membrane integrity. Membrane integrity may be compromised by biologic processes such as bio-fouling or grow-through or through a lack of proper maintenance. Because reverse osmosis is typically a “slow” process, drinking water storage is required for convenience.

Pathogens may enter storage waters from source water or contaminated system components (Singer, 1997). Reverse osmosis system manufacturers and installers have identified and in many cases successfully eliminated many of these problems with newer technologies. An estimate of the percentage of malfunctioning systems and the expected removal efficiencies of these affected systems is not known and in this example,100% of the *Cryptosporidium* oocysts is assumed to be removed. In similar comparison with the two treatment plants no system failures are considered in this analysis.

Lykins et al. (1991, p. 205) identified the initial cost of a reverse osmosis unit for a single tap to be between $500 and $800 and the installation cost to be between $70 and $150. Annual operation/maintenance and monitoring/administrative costs were estimated by Lykins et al. (1991) to be between $50-$100 and $15-$20, respectively. These values are presented as 1991 dollar estimates. In the interim these costs have increased as a result of inflation.

A.7.3. Sequale of Cryptosporidiosis in Healthy Immune Systems. As noted previously, cryptosporidiosis is almost always self-limiting in healthy adults. Nearly all of the deaths that occurred during the Milwaukee outbreak were in individuals with compromised immune systems. Assuming all of the oocysts are removed via the in-home reverse osmosis systems and no exposures occur to immunocompromised individuals, the probability of *Cryptosporidium* infection and death by cryptosporidiosis is so low that it is essentially 0. It is assumed in this application that no disease outbreaks will result in death.

Summary of Inputs	
Sequale of cryptosporidiosis	Disease is self-limiting in healthy adults; no deaths occur in
Cryptosporidium removal via reverse osmosis	100%
Initial costs of in-home treatment system (Based on a 1991 cost estimate)	$570-$950
Annual costs of in-home treatment system (Based on a 1991 cost estimate)	$65-$120

FIGURE A.1-1
Distribution of Public Water Systems by System Type and Source Water

FIGURE A.1-2
Representative Structures of DBPs

FIGURE A.1-3
Important Drinking Water Chemicals and Contaminants

FIGURE A.1-4
Typical Surface Water Treatment System

FIGURE A.1-5
Cross Section of Surface Water Treatment Train

FIGURE A.1-6
EPA Pilot Plant Configuration

FIGURE A.1-7
Schematic of Conventional Water Treatment Train

FIGURE A.1-8
Schematic of Conventional Water Treatment Train with Pre-Ozonation

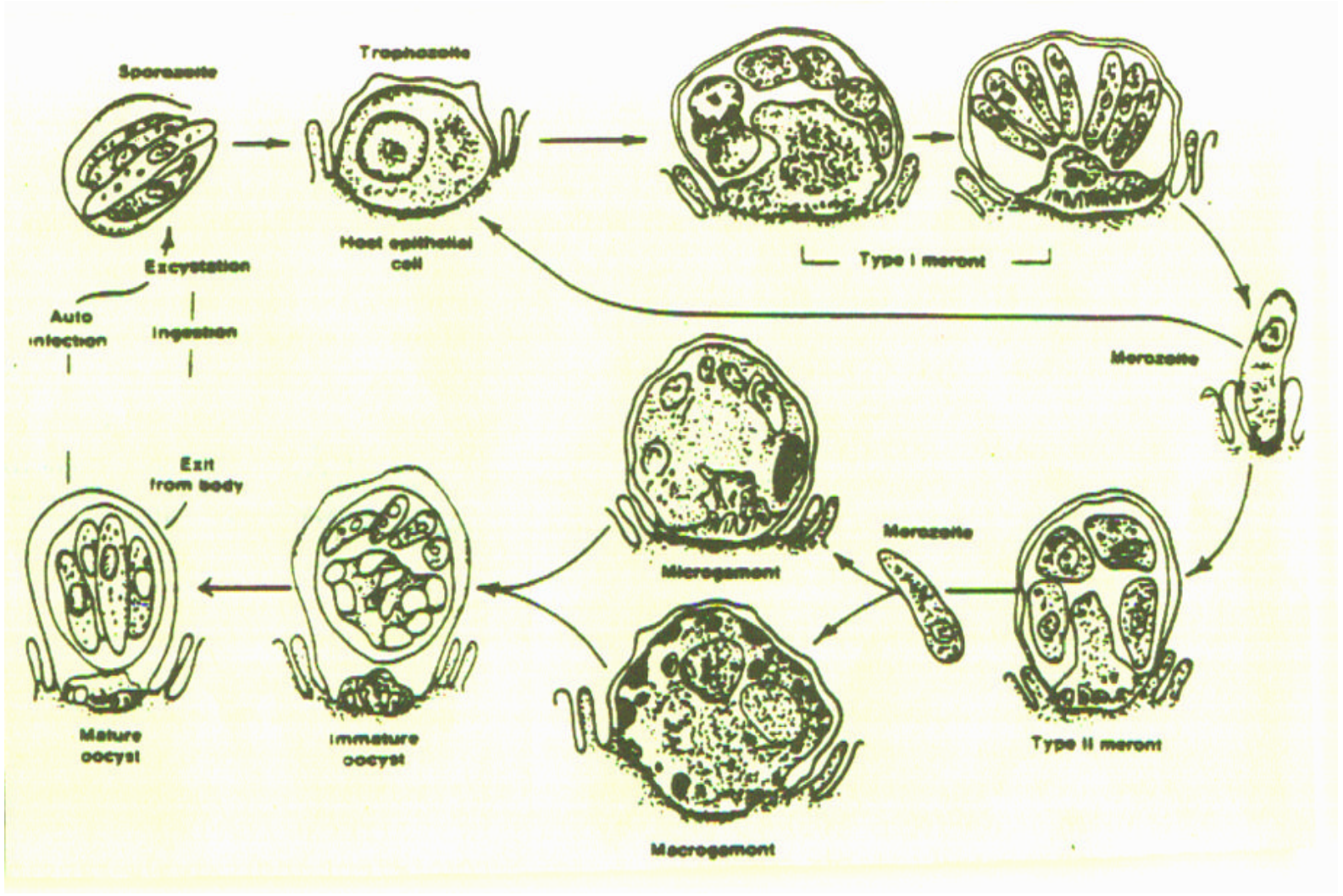

FIGURE A.2-1

Life Cycle of Cryptosporidium
Source: Fayer, 1997

FIGURE A-4-1
Selecting DBPs for Consideration

FIGURE A-4-2
Some Factors Impacting DBP Formation

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